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Contents

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FURTHER STUDIES ON THE AGGLUTINATION REACTION IN CHRONIC ARTHRITIS [1]

By EDITH E NICHOLLS and WENDELL J STAINSBY

(From the Department of Medicine of Cornell University Medical College, the Second (Cornell) Medical Division of Bellevue Hospital and the New York Hospital New York City)

The present authors (1), collaborating with Cecil, reported, in 1929, the isolation of streptococci from the blood and joints of patients having rheumatoid arthritis A large percentage of the organisms thus recovered were culturally and biologically similar, and presented the appearance of hemolytic streptococci on blood agar plates For convenience, these organisms were designated as "typical strains"

In later communications the authors reported, with Cecil (2), and alone (3, 4), that the serums of a high percentage of patients showing well developed signs of rheumatoid arthritis gave strong agglutination reactions with "typical strain" streptococci The same serums gave positive agglutination results with a few hemolytic streptococci from sources other than arthritis, but whenever such reactions took place the organisms were culturally and biologically indistinguishable from "typical strain" streptococci With other bacterial antigens, however, including indifferent and green producing streptococci, pneumococci, staphylococci and colon bacilli they gave little or no agglutination Likewise, negative or insignificant reactions were obtained when the serums of a large number of normal individuals and of patients with other diseases were tested for agglutinations with "typical strain" streptococci Among these control serums were 79 from rheumatic fever and 16 from osteoarthritis As a result of this study, the authors concluded that the serums of a high percentage of patients with rheumatoid arthritis gave strong agglutination reactions with a biologically specific type of hemolytic streptococcus Gray and Gowen (5), and Dawson, Olmstead and Boots (6) have in the main confirmed these observations

THE PRESENT STUDY

In order to obtain further information concerning this phenomenon, routine agglutination tests were carried out with "typical strain ' AB13 on the serums of all patients admitted to the arthritis department of the

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Cornell Clinic during a two-year period   These tests were repeated, when possible, at intervals throughout the patient's period of observation The purpose of this paper is to present an analysis of the findings in this study   Unless otherwise stated, all agglutination results reported refer to those at the time of the patient's first visit to the Clinic

Seven hundred and thirty-three cases are included—a few had to be discarded either because a diagnosis could not be made or because the patients were suffering from diseases other than arthritis   The incidence of each disease is given in Table 1

<div align="center">

TABLE 1

Tabulation of diseases studied

Disease	Number of cases
Rheumatoid arthritis	613
Ostearthritis	44
Monarticular arthritis	41
Rheumatic fever	14
Gonococcus arthritis	16
Gout	4
Intermittent hydrarthrosis	1
Total	733

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AGGLUTINATION TECHNIC

The method of performing the agglutination test was similar to that previously described (3) and so need not be explained in detail   No dilutions were made beyond 1 5120   Each test was controlled with a serum that was known to possess no streptococcus agglutinins and with a tube containing 0 5 cc of broth and 0 5 cc of culture   All agglutinations were heated in a water bath at 56° C for two hours   The tubes were then placed in a refrigerator and readings were made the following morning   The last dilution in which definite clumping of the bacteria could be detected by the naked eye was recorded as the agglutination titer

The agglutination tests on each specimen were carried out on two consecutive days and if the readings thus obtained did not correspond, the procedure was repeated until the titer was accurately ascertained

Occasionally the antigen showed a tendency to become granular This proclivity was usually overcome by daily transferring the culture to fresh blood broth mediums over a period of several days   Subculturing in broth containing 0 2 per cent disodium hydrogen phosphate, in place of the 1 5 sodium chloride, was also found to encourage diffuse growth of the organisms

RHEUMATOID ARTHRITIS

Six hundred and thirteen patients were diagnosed as suffering from rheumatoid arthritis—none were included in this group who had symptoms in one joint only

For convenience, the patients were allotted to one of three groups, according to the degree of joint involvement   The first group includes those who complained of pain in the joints, but failed to show any periarticular swelling, the symptoms were usually migratory in character and tenderness was frequently found   The second group is comprised of those who presented the usual picture of rheumatoid arthritis with charac teristic periarticular swelling, generally including fusiform fingers   The third group is similar to the second, but is made up of patients who were in a more advanced stage of the disease—deformities such as ulnar devia tion, and partial or complete ankylosis of one or more joints characterize this group   These three groups are indicated by +, ++, and +++, respectively, and the number and percentage of patients in each group are shown in Table 2

TABLE 2

Tabulation of patients with rheumatoid arthritis

Group	Number of patients	Per cent
+	310	50 6
++	241	39 3
+++	62	10 1
Total	613	100 0

In several tables and figures reference is made to mean agglutination titers   These group averages are computed by multiplying each figure representing a dilution of serum by the number of serums agglutinating at that titer, adding these products and dividing the sum by the total number of serums included in the group

In order that insignificant information be not included in the tables and figures, titers of 1 20 and 1 40 have been recorded as negative

Relation of incidence of disease to age of patient

In Figure 1 the age incidence of patients belonging to each of the three groups (+, ++, +++) is indicated   Only one patient under ten years of age was seen with rheumatoid arthritis, and only seven, in the second decade of life, while there was a sharp falling off after sixty years of age   That rheumatoid arthritis is chiefly a disease of middle life is clearly shown in Figure 1   The small proportion over 60 years of age may be due in part to the failure of patients at this age to seek medical advice, but no such reasoning is applicable to the low incidence in patients of twenty years of age or under   It must, therefore, be concluded that rheumatoid arthritis is a relatively rare disease during the first two dec ades of life   While the three curves are nearly parallel, there is a distinct tendency in the + group to mark its maximum incidence at an earlier age than do the others
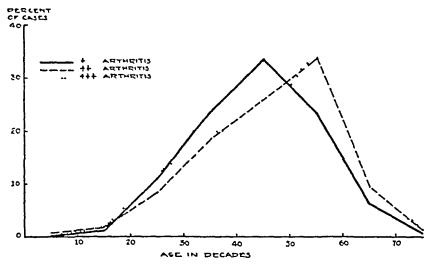
FIG 1   RELATIONSHIP OF INCIDENCE OF DISEASE TO AGE OF PATIENTS IN
RHEUMATOID ARTHRITIS (613 CASES)

Relation of agglutination titer to degree of joint involvement

In a previous study of agglutination reactions with rheumatoid ar-
thritis serums a selection of patients with marked joint involvement was
used   Of the 110 serums then examined, 93 6 per cent showed definite
agglutination to a titer of 1   640 or more (3)   In the present study of
613 cases all stages of rheumatoid arthritis are represented   In Figure 2
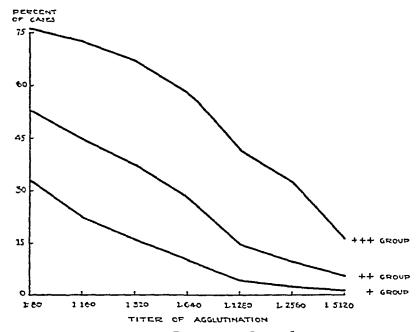
FIG 2   RELATION OF DEGREE OF JOINT INVOLVEMENT TO
TOTAL AGGLUTINATION (613 CASES)

is indicated the total agglutinations for each of the three groups   The highest degree of agglutination was found in the class with advanced rheumatoid arthritis $(+++)$, while a distinctly higher agglutination was found in the moderately advanced group than in the mild cases without swelling $(+)$ From these results it is evident that, on an average, the more advanced the joint involvement, the higher the titer of agglutination

Relation of agglutination titer to duration of disease

The agglutination titer in rheumatoid arthritis with relation to the duration of the disease is indicated in Table 3 and Figure 3   In 8 cases of disease duration of one month or less there was not the slightest evidence of the presence of agglutinins   In 45 cases of disease duration

TABLE 3

Results of agglutination tests in rheumatoid arthritis in relation to duration of disease

Duration	Cases	Neg ative	Titer of agglutination							Mean titer
			1 80	1 160	1 320	1 640	1 1280	1 2560	1 5120	
One month or under	8	8								0
Over 1 month to 3 months	45	30	6	5	2	1			1	1 171
Over 3 months to 6 months	66	31	5	6	5	11	3	2	3	1 520
Over 6 months to 1 year	92	49	14	4	9	8	1	3	4	1 426
Over 1 year to 3 years	126	62	10	5	12	16	8	7	6	1 591
Over 3 years to 5 years	88	42	11	8	5	9	6	4	3	1 486
Over 5 years	188	115	7	13	13	17	7	7	9	1 482

FIG 3   MEAN AGGLUTINATION TITER IN RHEUMATOID ARTHRITIS
IN RELATION TO DURATION OF DISEASE (613 CASES)

from one to three months the highest agglutination titer recorded with one exception was 1 640 and the mean was 1 171 In the group having the disease from three to six months, 3 showed an agglutination titer of 1 5120, while the average was 1 520 In the groups covering a disease duration of over six months little variation was noted, the line on the graph closely approaching a straight one A similar analysis, employing only cases in groups + + and + + +, revealed a curve similar to that when all three groups were used

From these results it is evident that it takes six months, on the average, for a patient to develop hemolytic streptococcus agglutinins to the maximum titer, but that aside from this initial period the duration of the disease plays no important part in the strength of the agglutination reaction Dawson, Olmstead and Boots (6), reporting a series of 153 cases, stated that the agglutination titer increased with the duration of the disease Our findings do not confirm this conclusion

Relation of agglutination titer to age of patient

In Table 4 and Figure 4 the results of the agglutination tests in rheumatoid arthritis are arranged in relation to the age of the patients A boy of seven with moderately advanced rheumatoid arthritis was the only patient under 10 years of age seen in the arthritis clinic His serum

TABLE 4

Results of agglutination tests in rheumatoid arthritis in relation to age of patient

Age	Cases	Negative	Titer of agglutination							Mean titer
			1 80	1 160	1 320	1 640	1 1280	1 2560	1 5120	
years										
Under 10	1					1				
10 to 19	7	4	1			2				1 194
20 to 29	62	34	8	3	4	4	2	2	5	1 616
30 to 39	129	64	18	9	9	9	9	7	4	1 476
40 to 49	184	108	10	12	16	21	3	5	9	1 457
50 to 59	174	94	12	15	13	19	9	6	6	1 444
60 to 69	50	30	4	2	3	5	2	3	1	1 403
70 or over	6	3				1	1	1		

showed an agglutination titer of 1 640 with "typical strain" streptococcus AB13 There were but 7 patients between ten and nineteen years of age—serums from 2 of these gave an agglutination titer of 1 640, from 1 a titer of 1 80, and from 4 there were negative findings, with a mean agglutination titer for the group of 1 194 The age period covering the third decade gave, on an average, the highest titer of agglutination, while in the periods following there was a slight gradual drop all the way to the last group which included patients seventy years of age or older

Dawson, Olmstead and Boots (6) reporting a sharply progressive in crease in the median agglutination titers of patients in the third, fourth and fifth decades, concluded that "the property of rheumatoid arthritis serum responsible for the agglutination of hemolytic streptococci is definitely related   to the age of the patient " Our results do not agree with their findings   When, however, groups +, + + and + + + of our series are plotted individually, group + + + tends to show increased titers of agglutination in the fourth, fifth and sixth decades   It seems probable then that the series of cases reported by Dawson, Olmstead and Boots is preponderantly made up of cases corresponding to those in group + + + of the present series   In the opinion of the writers, a more accurate impression of the relation of age to the titer of agglutination can be obtained from the study of the disease as a whole than from an analysis of certain of its phases
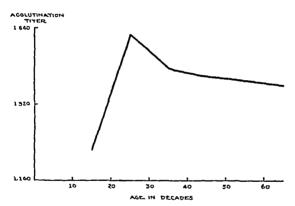
FIG 4   MEAN AGGLUTINATION TITER IN RHEUMATOID ARTHRITIS IN RELA TION TO AGE OF PATIENT (606 CASES BETWEEN THE AGES OF 10 AND 69)

In the present series, the number of patients in the first, second and eighth decades of life is too small to draw any definite conclusions con cerning their agglutination reactions with "typical strain" streptococci It seems probable, however, that in patients under twenty years of age the agglutination titer averages lower than in patients over that age   This impression receives support by the findings in a study of agglutinins in the serums of six patients in Bellevue Hospital suffering from Still's disease, a condition accepted by many as rheumatoid arthritis in early childhood Although the same technic was carried out, negative results were obtained in each case   It is doubtful if a lower titer in patients under twenty years of age has any significance because during that period rheumat

thritis is uncommon and when it does occur it assumes atypical forms, frequently resembling rheumatic fever

In summing up the relationship of the age of the patient to the agglutination titer, one is led to the conclusion that with the possible exception of the first two decades of life the age of the patient plays no important role in the strength of the agglutination reaction

Relation of agglutination titer to time of year

Clinically, it is generally recognized that in temperate climates patients with rheumatoid arthritis are usually at their worst in cold weather and at their best in hot weather   In order to ascertain if there is any seasonal variation in the agglutination titers in this disease Table 5 was prepared

TABLE 5

Results * *of agglutination tests in rheumatoid arthritis in relation to season of year*

Season	Cases	Neg ative	Titer of agglutination							Mean titer
			1 80	1 160	1 320	1 640	1 1280	1 2560	1 5120	
Winter	98	33	12	8	9	17	8	7	4	1 660
Spring	79	30	3	4	7	16	3	5	11	1 1074
Summer	46	30	3	2	1	4	1	2	3	1 548
Autumn	80	35	4	6	12	7	4	7	5	1 728

* Total of 303 cases—groups ++ and +++ only

Cases of arthritis in groups ++ and +++ were selected as patients in the more advanced stages appeared to show the greatest seasonal variation   In the table, "Winter" is used to designate the period from January 1 to March 31, "Spring," from April 1 to June 30, "Summer," from July 1 to September 30, and "Autumn," from October 1 to December 31   The findings are interesting   The mean agglutination titer of 79 patients studied in the spring was 1   1074, while that of 46 studied in the summer was only 1   548   The mean titers during the autumn and winter were 1   728 and 1   660 respectively   Due to the small number of cases in this series and the distribution of the agglutination titers entering into the means, the degree of probability of chance occurrence of the differences between the means is too high to warrant any definite conclusions   Nevertheless the results are at least suggestive that on the average the agglutination titers are higher in the spring and lower in the summer than at other seasons of the year

Relation of titer of agglutination to clinical changes

Initial agglutination tests on all patients were repeated at irregular intervals throughout each period of observation   Condensed protocols of those subjected to the test four or more times appear in Table 6   The

TABLE 6

Patients with repeated agglutinations

Patient number	Sex	Age	Duration of disease	Joint involvement	Date	Titer	Date	Titer	Date	Titer	Date	Titer	Date	Titer	Date	Titer	Date	Titer	Date	Titer	Clinical condition during observation
1	M	4	1 year	+++	12/29/30	1:5120	1/13/31	1:5120	7/9/31	1:5120	4/29/31	1:5120	7/3/31	1:1280	4/29/31	1:5120	5/19/31	1:5120			Unchanged
2	F	40	3 years	+++	4/10/30	1:510	2/1/31	1:1280	4/13/31	1:2560	4/13/31	1:5120			5/23/31	1:5120					Gradually worse
3	M	36	6 months	+++	10/20/31	1:610	3/1/31	1:5120	4/27/31	1:1280	7/1/31	1:1280									Unchanged
4	F	53	3 years	+++	4/15/31	1:640	8/26/31	1:390	10/9/31	1:640	1/6/32	1:320									Unchanged
5	F	45	6 years	+++	1/12/31	1:640	4/29/31	1:390	8/25/31	1:2560	10/9/31	1:640									Discharged
6	F	48	6 months	++	1/18/31	1:160	8/26/31	1:5120	6/29/31	1:5120	10/9/31	1:560									Unchanged
7	F	57	2 months	++	2/7/31	1:160	6/10/31	1:1280	9/30/31	1:1280	6/1/32	1:320									Unchanged
8	M	53	15 years	+	9/0/31	1:2560	9/23/31	1:320	6/1/32	1:320	7/16/31	1:640	7/14/32	1:640							Unchanged
9	F	63	5 years	+++	1/29/31	1:840	10/7/31	1:80	10/14/31	1:80	7/16/31	1:640									Possibly slightly worse
10	M	65	1 month	++	10/7/31	1:3 0	1/6/3	1:.51^0	2/4/32	1:2560	4/13/32	1:2560	4/13/32	1:3 0 0							Unchanged
11	F	69	3 months	+++	4/17/31	neg.	10/2/31	1:2.0	10/23/31	1:5120	4/8/32	neg.	5/25/3^?	1:3 0 0							Unchanged
12	F	45	8 years	+	6/3/31	1:160	10/9/31	1:37 0	10/23/31	1:1160	3/4/32	1:160									Gradually worse
13	F	33	4 months	++	1/18/31	1:2560	8/26/31	1:2560	9/27/31	1:1320	3/30/32	1:640	5/13/32	neg.							Irregular course. In general worse
14	F	33	11 years	+	5/18/31	1:7350	9/23/31	1:640	10/7/31	1:320	11/25/31	1:640									Irregular course. In general unchanged
15	M	20	2 years	++	1/18/31	neg	5/1/31	neg.	10/30/31	neg.	11/25/31	neg.	3/16/3	neg.							
16	M	36	4 years	+++	4/22/31	1:51^0	8/14/31	1:100	10/16/31	1:640	2/26/32	1:320	9/5/32	neg.	5/19/32	neg.			Irregular but definite improvement
17	F	31	7 years	++	12/10/31	1:1280	4/1/31	1:51^0	6/17/31	1:80	4/13/32	neg.	9/5/31	neg.							Slight improvement
18	M	41	1 month	++	12/11/31	1:640	2/1/31	1:3 0	4/29/31	1:40	6/16/31	1:80	6/24/31	neg.							Gradually improved	Discharged cured
19	F	0	10 weeks	+++	12/20/30	neg	12/29/30	1:640	1/13/31	1:1280	7/7/31	1:80	8/31	neg.	3/11/3	neg	Gradually improved	Discharged cured
20	M	37	3 months	++	7/10/31	1:640	12/22/30	1:640	11/20/31	neg.	6/24/31	neg.	5/13/31	neg.							Gradually improved
21	F	28	6 months	++	4/6/31	1:560	9/22/31	1:370	11/20/31	1:560	2/5/32	neg.	4/1/31	1:320	4/7/32?	neg.	Gradually improved Cured (4/15/32?)
22	F	28	3 years	+++	2/11/31	1:1280	4/1/31	1:1^90	8/9/31	1:3 0	10/23/31	1:640	2/6/32	1:320			Constant improvement beginning spring 1931
23	F	72	4 years	+++	1/18/31	1:1280	4/27/31	1:5120	10/23/31	neg.	1/7/33	neg	2/9/31	1:80			Gradually improved. Discharged cured
6	F	31	3 years		7/22/31	1:1^80	3/1/31	neg.	6/4/31	neg.	1/4/3	neg	4/5/32	neg	4/7/7/3?	neg	Marked improvement beginning spring 1932
27	M	30	1 year	+	12/10/30	1:80	10/4/31	neg	7/4/31	neg.	9/7/31	neg.	11/5/31	neg	3/2/32	neg.	Irregular course. Improved last 6 months
28	M	33	2 years		1/18/31	1:1280	3/4/31	1:640	5/29/31	1:1 80	4/8/32	1:320							Gradually improved
'9	F	30	1 year	+	1/10/30	1:80	6/10/31	1:1280	5/18/3^?	1:80	9/0/31	1:80					Improvement beginning summer 1932
30	M	40	4 months	+	1/7/31	1:640	6/3/31	1:51^0	9/0/31	1:2560	4/6/32	1:160					Severe exacerbations during spring of 1931 5/16/32 condition about as on admission. Worse during first 6 months. 4/6/32 much improved

findings on one patient who had but three examinations were included because of their special interest

Patients 1 to 15, inclusive, showed no appreciable change in clinical condition throughout the observation period   In these cases the titers of agglutination evidenced a strong tendency to remain at a constant level

In contrast to the findings on the fifteen patients mentioned are those of patients 16 to 28 who showed definite clinical improvement, sometimes with complete recovery   In this group the serums tended either to agglutinate at lower levels or else to completely disappear, but generally the strength of the agglutination did not show signs of diminishing until some weeks or months after the patient began to improve clinically   Patient 19 illustrates this proclivity very well   This patient with a history of polyarthritis of only ten weeks standing, whose illness was running a subacute course, presented the typical features of rheumatoid arthritis at the time of his first visit   An agglutination test at that time (December 20, 1930) gave negative results   The patient showed constant improvement dating from the time of his first visit and was entirely free from signs and symptoms on January 13, 1931   In spite of this clinical improvement, the agglutination titer continued to rise for one month and only began to fall after the patient was entirely free from signs and symptoms of the disease

Patient 23, with a more chronic form of the disease, illustrates the same process   With a history of rheumatoid arthritis of three years duration she showed marked improvement from the Spring of 1931, yet it was several months before the agglutination titer began to diminish and not until one year later that the agglutinins disappeared completely

Patients 29 and 30 illustrate the tendency of the agglutination titers to rise and fall with changes in the clinical condition   At their first visit both of these patients presented the picture of a very mild and early rheumatoid arthritis   While under treatment their conditions gradually became very much worse, with a definite increase in the agglutinin content of their serums   At a still later period the arthritis showed definite abatement, which condition was followed by a diminution of streptococcus agglutinins in the serums, evidenced by a lower agglutination titer

In summing up the results of repeated agglutination reactions in rheumatoid arthritis, it is apparent that the agglutinin content in the patient's serum is related to his clinical condition, tending to increase gradually during the development of the disease, and to decrease gradually with the recovery of the patient   There seems also to be considerable individual variation in the time required for clinical change to be manifested in the agglutination reactions

OTHER FORMS OF ARTHRITIS

The results of agglutination tests in other forms of arthritis seen during the period of this study are presented in Table 7

TABLE 7

Results of agglutination tests in other forms of arthritis

Disease	Negative	Titer of agglutination							Cases
		1 80	1 160	1 320	1 640	1 1280	1 2560	1 5120	
Ostearthritis	38	6							44
Monarticular arthritis	33	4	1	2	1				41
Rheumatic fever	11	1	1	1					14
Gonococcus arthritis	14	2							16
Gout	4								4
Intermittent hydrarthrosis	1								1

Forty four patients were diagnosed as suffering from ostearthritis—special care being taken not to confuse true ostearthritis with rheumatoid arthritis showing secondary ostearthritic changes   All of these patients were over forty years of age and not one serum of the 44 gave an agglutination reaction to a titer higher than 1 80, while 38 were completely negative

In the group classified as monarticular arthritis are included, for convenience, cases of spondylitis   Probably some of these 41 cases were of infectious origin of the rheumatoid arthritis type, but because of the uncertainty of diagnosis they were segregated in this way, 33 gave negative results to agglutination tests, while the highest titer recorded by the remaining 8 was 1   640

Fourteen patients suffered from rheumatic fever   Eleven gave negative agglutinations, while the highest titer recorded was 1 320

Sixteen cases of gonococcus arthritis were seen   A diagnosis made from the history of the association of gonococcus urethritis with the onset of joint symptoms was frequently confirmed by positive findings in a complement fixation test   The highest agglutination recorded for this group was at a titer of 1 80

Four cases of gout and one of intermittent hydrarthrosis gave completely negative reactions

SUMMARY AND DISCUSSION

Seven hundred and thirty three patients with chronic arthritis were studied by agglutination tests with "typical strain" streptococcus AB13

In this aggregation rheumatoid arthritis was the predominating disease, as 613 of the patients were suffering from that affection   The age incidence in this group was an interesting feature   Only one patient under ten years of age was seen and there was a gradual increase up to and including the sixth decade, following which there was a sharp drop   The maximum incidence of patients without joint swelling occurred at a somewhat earlier age than the highest number with joint swelling

A previous article by the present authors showed that the serums of a high percentage of selected patients with well-developed rheumatoid arthritis gave strong agglutination reactions with a specific type of hemolytic streptococcus   In the present study, all types of rheumatoid arthritis are included and a more detailed analysis of the results has been made

Apparently, the degree of joint involvement is an important factor in the strength of the agglutination reaction, as the tests proved that the more marked the joint involvement, the higher the agglutination titer Moreover, as the amount of joint involvement is generally considered to be commensurate with the severity of the disease, it does not seem unfair to assume that as the arthritis increases in severity more evidence is found of streptococcus agglutinins in the serum

An analysis of the agglutination reaction in relation to the duration of the disease reveals the fact that it takes several weeks following the onset of the disease for the streptococcus agglutinins to become apparent in the serum, and that the maximum titer is not obtained until an average of six months has elapsed   Except in this relation, the duration of the disease appears to play no important role in the agglutination phenomenon

The age of the patient, likewise, showed little effect on the strength of the agglutination reaction   An exception must be made, however, in the first two decades of life   Although one patient seven years of age gave a definite agglutination to a titer of 1 640, the tendency was for the titer in subjects of this age to be weaker and more irregular   As has already been pointed out, little significance can be attached to this observation because such patients seldom manifest the typical clinical picture of rheumatoid arthritis, their signs and symptoms frequently resembling those of rheumatic fever, a disease that does not give positive results in tests with "typical strain" streptococci

Of particular significance are the findings in patients subjected to repeated agglutination tests extending over a period of months or years The results of these tests are remarkably consistent as the serums of patients in whom the disease appears to be stationary continue to give similar agglutination titers throughout the period of observation   In patients fortunate enough to show marked improvement or complete recovery, on the other hand, the streptococcus agglutinins either diminish or completely disappear from the blood   Sometimes this is accomplished in a few weeks, sometimes it takes months or even years

With the information at hand concerning the agglutination reaction in rheumatoid arthritis, it would seem justifiable to consider the nature of this phenomenon   Unfortunately, we do not have a chronic disease that affords a comparison and must, perforce, compare it with an acute disease, such as typhoid fever   In typhoid the Widal reaction is considered to be an immunological one   Following the onset of the disease,

there is a definite interval of several days before a positive typhoid ag glutination can be obtained   In the succeeding days or weeks the strength of the reaction tends to increase, while on recovery of the patient it slowly diminishes and in the course of several months or years even tually disappears   In rheumatoid arthritis a similar situation exists   The only essential factor in which it differs from that in typhoid fever is the time element   In typhoid the agglutination changes are apparent after periods of days—in arthritis, after periods of weeks or months   This would seem to be a natural disparity between the processes in an acute and a chronic disease   Although not irrefutably proven, these findings afford strong evidence that the presence of streptococcus agglutinins in such high titers is a true immunological response to a bacterial invader similar in all essential respects to that seen in typhoid fever

In contrast to the positive results obtained from agglutination tests in rheumatoid arthritis are the negative results in ostearthritis, gonococcus arthritis, rheumatic fever, and gout   A knowledge of this reaction is of practical value in differential diagnosis   A positive reaction at titers of 1 320 or higher is indicative of rheumatoid arthritis but the reverse does not hold true because a small percentage of patients even though they present the typical picture of rheumatoid arthritis have serums that give negative agglutination results with "typical strain" streptococci

CONCLUSIONS

1 A high percentage of patients with rheumatoid arthritis give posi tive agglutination reactions to a specific type of hemolytic streptococcus

2 In patients with advanced joint involvement higher average titers are obtained than in those with less involvement

3 The duration of the disease and the age of the patient play unim portant roles in the strength of the agglutination

4 Following the onset of the disease, there is a gradual increase in the agglutination titer which reaches its maximum in 6 months, on the aver age, while following recovery of the patient the agglutinins tend to diminish and eventually to disappear

5 This reaction of serum in rheumatoid arthritis appears to be a true immunological response

6 Other forms of arthritis do not give positive results in agglutination tests with typical strain streptococci

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GASTRO-INTESTINAL STUDIES   II   PANCREATIC ENZYMES IN PERNICIOUS ANEMIA

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In a previous article (1) an attempt was made to correlate the findings in the gastric juice of patients having pernicious anemia with the clinical condition of the patients, the degree of central nervous system involvement present, and the maintenance dose of liver extract   Little or no correlation could be found, therefore, this study of the duodenal contents of patients having pernicious anemia was undertaken   The work of Castle and his associates (2) also indicated that additional studies on the digestion of proteins in cases of pernicious anemia should be carried out

Ehrmann and Lederer (3) (1908) were the first to describe the results of duodenal analyses in cases of achylia gastrica, normal or slightly higher values being found by them   Einhorn (4) (1910) reported the results of the examination of 7 cases of achylia gastrica, in which 4 had normal trypsin values, 1 a low value, and 2 a complete absence of trypsin   In 1914 he reported (5) two cases of achylia gastrica with chronic pancreatitis in which there was an absence of trypsin but in which the other duodenal enzymes were approximately normal   He found (6) high enzymatic activity in the duodenal contents of 3 other cases with achylia gastrica, in 1918

Crohn (7), in 1913, recorded a case of gastric achylia in which there were normal amounts of all duodenal enzymes except pancreatic rennin In 1915, he (8) stated that "in my present series, consisting of over 120 cases, 103 cases are regarded as presenting normal pancreatic function, these include many pathological conditions, gastric lesions, benign and malignant, achylia gastrica, organic syphilis, exophthalmic goitre, secondary and primary anemias, malignant growths of various organs, diabetes mellitus, etc"   Chace and Myers (9), in 1913, reported 1 case of pernicious anemia and 3 other cases of achylia gastrica in which there were normal amounts of duodenal enzymes   White (10), in 1916, studied another case of pernicious anemia and 3 other cases of achylia gastrica, all four having normal or high pancreatic enzyme values   In 1922, McClure and Jones (11), after studying two cases of pernicious anemia in severe relapse and two other cases of achylia gastrica, concluded that "in achylia gastrica and pernicious anemia no abnormalities in activity of

the external secretory function of the pancreas were demonstrable, as measured by the enzyme determination of the duodenal contents " In 1922, Roth and Sternberg (12) reported 6 cases of achylia gastrica in which the pancreatic enzymes were present   Kahn (13) also, in 1923, described one case of sprue with gastric and pancreatic achylia   The pancreatic achylia disappeared on proper diet, although the gastric achylia persisted   McClure, Montague, and Mortimer (14), in 1924, found another case of achylia gastrica in which the pancreas functioned normally both as to enzymes and alkaline fluid   Silverman and Denis (15), in 1924, observed one case of achylia gastrica with approximately normal duodenal enzymes   Piersol and Bockus (16) recorded the findings in 3 cases of achylia gastrica in 1925   In two of these cases there was a rather low trypsin activity, while, in the third, trypsin was absent in one examination and present in a higher percentage than normal in another Landau et al (17), in 1926, reported 4 cases of pernicious anemia in which there was both gastric and pancreatic achylia   By 1929 Landau and Glass (18) had collected 9 cases of pernicious anemia with both gastric and pancreatic achylia   (Four of these had been reported in their previous article)   They stated that, since the introduction of duodenal and gastric analyses, they had done daily examinations and these nine cases were the only ones in which both gastric and pancreatic achylia was found

Martin (19), in 1927, found normal amounts of trypsin and diastase in the duodenal contents of two cases of achylia gastrica   Okada et al (20), in 1929, after studying the pancreatic enzymes of 7 cases of achylia gastrica stated that "evidence that the pancreatic secretion was disturbed as the consequence of disturbed gastric secretion was not found "   Cheney and Niemand (21), in 1932, stated that fasting gastric contents contain approximately the same concentration of pancreatic enzymes as the fasting duodenal contents   They made determinations of trypsin on the fasting gastric contents of 10 cases of pernicious anemia in relapse and in 9 of these there was an absence of trypsin, while, in the other, only a small amount of trypsin was found   In 60 other cases (mainly achylias) normal amounts of trypsin were found in the fasting gastric contents

In the series of experiments reported in this paper, we have attempted not only to evaluate the enzymatic activity of the pancreas, but to determine, as well, the ability of the duodenum to activate the trypsinogen secreted by the pancreas   To do this we have ascertained the tryptic power of the duodenal contents before and after incubation with enterokinase prepared from the duodenal mucosa of hogs

MATERIAL AND METHODS

Five young, healthy adults without evidence of disease and having had previous normal gastric analyses were used as controls   All of the 22 cases of

pernicious anemia were typical clinically and hematologically and had had previous complete gastric analyses In Table I the clinical and hematological findings in the 22 cases of pernicious anemia are recorded The results of the gastric analyses in 16 of the 22 patients have been reported previously (1)

TABLE I

The clinical and hematological status of the 22 cases of pernicious anemia

Case number	Age	Red blood cells	Hemoglobin (Newcomer)	Daily maintenance dose of liver extract no 343—derived from grams liver	Central nervous system involvement
	years	*millions per c mm*	*per cent*		
1	78	2 90	65		Moderate
2	62	4 89	78	Intravenous*	Advanced
3	59	4 86	84	300	Advanced
4	58	5 19	92	300	Advanced
5	54	4 54	84	Intravenous	Early
6	46	2 99	49		Early
7	55	5 64	94	300	Advanced
8	71	4 14	86	50	Early
9	49	4 93	73		Advanced†
10	58	5 10	78	Intravenous	Early
11	46	4 63	69	Intramuscular	Moderate†
12	72	5 60	86	Intravenous	Advanced
13	64	4 68	83	Intramuscular	Advanced†
14	66	5 31	110	300	Advanced
15	42	5 65	101	300	Early
16	54	5 28	83	Intravenous	None
17	48	4 73	88		Early
18	58	5 08	89	400	Moderate
19	54	1 15	25		Advanced
20	48	4 95	84	400	Early
21	62	1 73	31		Advanced
22	58	1 16	27		Advanced

* Those patients now receiving intravenous or intramuscular liver extract at weekly intervals had previously been unable to maintain the blood at normal levels while receiving at least the amount of liver extract derived from 300 grams of whole liver daily by mouth

† Patients showing recent improvement in neurological conditions

No food or drink was given to the subjects between the evening meal and the morning of the test Early in the morning the fasting gastric contents were removed by means of a Rehfuss tube while the subject was in a semi recumbent position The subject then swallowed the tube to approximately the 75 cm mark and was turned onto the right side After the subject had rested in this position for from $\frac{1}{2}$ to $\frac{3}{4}$ of an hour, an attempt was made to localize the position of the tube To localize the tube the following procedures were followed

1 Auscultation was performed over the epigastrium during the injection of air into the tube (As stated by Richards (22), if the tip of the tube was in the stomach a loud cavernous sound was transmitted to the ear of the examiner but if it was in the duodenum, a more distant, muffled, high pitched sound was heard)

2 Sensation of the subject on the injection of the air was noted   (Many of the patients noticed a distinct difference in sensation when the air was injected into the duodenum   The injected air was felt much deeper and very quickly could be felt passing through the intestines)

3 The rapid disappearance of the acid injected was observed

4 Persistence of deep bile color to the fluid on removal was noted

If the above requirements were fulfilled, it was thought that the tip of the tube was in the duodenum and that the fluoroscope was not absolutely necessary to verify the position of the tube   Fluoroscopic examination probably would have helped in several instances in passing the tube into the duodenum, as, occasionally, it was necessary to wait three hours or longer and to change the position of the subject and the tube many times before the tube dropped into the duodenum   On a few occasions the examination was unsuccessful as the tube would not pass into the duodenum

After localizing the tube, 30 cc of 0 2 per cent hydrochloric acid was injected into the tube   One minute later an attempt was made to remove the acid   If most of the acid had disappeared at that time, continuous suction was applied to the tube after five minutes   Two 30 minute samples of duodenal secretions were collected

The duodenal or gastric juice was measured in a graduated cylinder and filtered through paper   The color was noted and the pH was determined colorimetrically   Only the results of those samples which were neutral or alkaline were considered to be of value   Silverman and Denis (15) and Wadsworth and Aaron (23) showed the value of this precaution   Our own experience has also taught us that acid duodenal contents gave much lower enzyme values

Amylase and lipase were determined by the methods described by McClure, Wetmore, and Reynolds (24)   However, in the lipase determination, 1 cc of duodenal juice was diluted to 5 cc with 0 33 molar phosphate buffer instead of to 50 cc   Our lower lipase values may be due to the difference in the stimulus used or in the cottonseed oil emulsion

To determine the tryptic activity of the duodenal juice, 1 cc of the juice was diluted to 10 cc with distilled water, and 5 cc was used for analyses by the method of Koch and Helmer (25) described below   The total tryptic content (trypsin plus trypsinogen) was determined by adding 1 cc of a 2 per cent solution of enterokinase to 1 cc of duodenal juice, making the total volume to 10 cc with distilled water, and incubating for 30 minutes at 40° C   Five cc of the activated solution was then used for the analysis of its tryptic power   The enterokinase was prepared from duodenal mucous membrane of hogs   The mucous membrane previously had been dehydrated and defatted by means of acetone and ether

The details of the method for the determination of trypsin are as follows The casein solution, which was used as a substrate, was prepared by shaking 75 grams of Merck's casein (according to Hammarsten) with 500 cc of distilled water in a 2 liter flask until the casein was in a finely divided state   Then 500 cc of 0 8 per cent sodium carbonate was added and the mixture shaken until the casein was dissolved   If preserved with toluene, the solution will keep several days in the ice box

For the determination, 80 cc of the casein solution was measured into an Erlenmeyer flask of 125 cc capacity, and enough 0 4 per cent sodium carbonate was added so that the total volume was 100 cc upon the addition of the substance to be tested   The casein solution was then allowed to come to 40° C and the trypsin was added   The mixture was stirred well to insure homogeneity, and 25 cc was pipetted into a small beaker or flask containing 3 6 cc of

normal acetic acid and about 1 gram of talc   (It is important to stir vigorously while adding the casein to the acid to insure a good precipitation)   The remaining solution was allowed to digest for 4 hours at 40° C   The 25 cc. of the solution above served as a blank   The precipitated casein was removed by filtration through a good quantitative paper and the refractive index was read at 25° C by means of a Bausch and Lomb immersion refractometer

At the end of the digestion period, exactly 4 hours, the flask was removed from the incubator and, again, 25 cc of the solution was pipetted off and the undigested casein precipitated as before   The difference in the refractive index of the blank and the 4 hour filtrate was the index of the amount of digestion   The refractive index changes were recorded in scale readings of the refractometer   Since the filtrate from the casein precipitation was water clear, no difficulty was encountered in securing constant readings with the refractometer

In order to have a standard with which to compare the tryptic activity a standard curve was made by determining the change in refractive index caused by quantities of U S P pancreatin, ranging from 1 mgm to 20 mgm per 100 cc of casein solution   The refractive index changes caused by known concentrations of a 0 4 per cent sodium carbonate solution of U S P pancreatin are shown in Table II, and the data from Table II are plotted in Chart 1

TABLE II

Change in refractive index readings at 25° C produced by known concentrations of U S P pancreatin

Pancreatin	Change in refractive index
	scale readings
	immersion
mgm	*refractometer*
1	0 72
2	1 20
4	2 10
6	3 35
8	4 18
10	5 36
15	7 45
20	9 17

To rule out the possibility of pepsin interfering with the trypsin determination, 5 cc of normal gastric juice with a high pepsin content was allowed to act on casein in the manner outlined above   The gastric juice had no proteolytic activity under these conditions

RESULTS IN NORMAL SUBJECTS

In Table III are tabulated the results in normal subjects   The fasting gastric contents all contained a trace of trypsin and lipase   In the samples with acid reaction the amylase was entirely absent   The one specimen which was alkaline had considerable amylolytic power, due, no doubt, to the presence of saliva   This sample also had the greatest tryptic activity

In the duodenal specimens in which the reaction was neutral or alkaline, the results were quite uniform   Again it was noted that the acid reaction completely inactivated the amylolytic enzyme and mar¹ ⁿᵈ¹
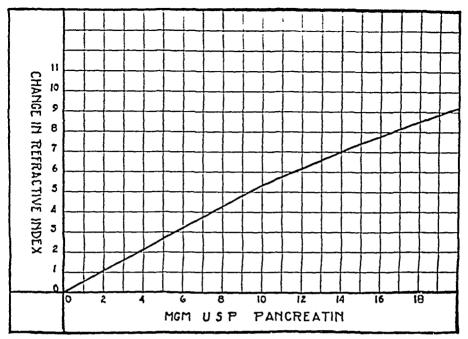
CHART 1 CHANGE IN REFRACTIVE INDEX READINGS AT 25° C PRODUCED
BY KNOWN CONCENTRATIONS OF U S P PANCREATIN

TABLE III

*The volume, color, pH, trypsin before and after activation with enterokinase, lipase,
and amylase in the fasting gastric juice and in the duodenal contents following acid stimu
lation in normal individuals*

Case num ber	Date	Specimen	Vol ume	Color	pH	Tryp- sin	Tryp- sin and tryp sin- ogen	Lip ase	Amyl ase
	1932		*cc*			*mgm per cc*	*mgm per cc*	*cc N/10 NaOH*	*mgm glu cose*
1	September 6	Fasting gastric	28	Negative	1 8	0 3	1 8	0 0	0 0
		1st half hour	80	Yellow	7 4	13 3	27 5	3 2	2 1
		2d half hour	30	Yellow	7 4	17 0	32 9	3 5	2 9
2	September 8	Fasting gastric	23	Bile	7 6	2 0	3 9	0 2	1 3
		1st half hour	95	Yellow	7 8	14 2	27 7	2 7	1 0
		2d half hour	12	Yellow	7 8	23 8	42 7	4 2	2 6
3	September 22	Fasting gastric	35	Bile	1 3	1 0	1 0	0 2	0 0
		1st half hour	64	Yellow	7 6	16 3	20 6	2 6	1 6
		2d half hour	46	Yellow	4 7	6 5	13 0	1 6	0 0
4	October 6	Fasting gastric	17	Negative	2 2	1 8	2 2	0 2	0 0
		1st half hour	110	Amber	8 0	13 2	25 2	2 8	0 9
		2d half hour	65	Amber	8 3	15 9	31 0	4 3	1 5
5	October 11	Fasting gastric	70	Bile	2 0	1 0	1 2	0 1	0 9
		1st half hour							
		2d half hour	107	Yellow	7 0	12 2	22 0	2 8	0 9

creased the lipolytic and tryptic activity   It was interesting to observe the relatively large amount of trypsinogen that was present in the normal duodenal contents   After activation, the tryptic power of the juice was increased 83 per cent   We believe that the determination of the tryptic power after activation with enterokinase may offer a more accurate index of pancreatic function, since at least two factors must be considered as playing a role in determining the amount of tryptic activity in the duodenal juice—namely, the ability of the pancreas to secrete trypsinogen and the ability of duodenal mucosa to supply enterokinase to activate it

RESULTS IN PERNICIOUS ANEMIA

In Table IV the results of the analyses of the gastric and duodenal contents in the 22 cases of pernicious anemia are recorded   The fasting gastric samples contained only small amounts of trypsin and lipase, except in the samples containing bile where there was a noticeable increase in the amount of these enzymes in most cases   Many of the fasting gastric samples contained a greater amount of amylase than the duodenal samples   This was undoubtedly due to the presence of saliva in the fasting gastric contents, since patients with pernicious anemia have no acid in the fasting gastric contents to destroy the amylase of the saliva

There was an increase in tryptic and lipolytic activity over the fasting gastric findings in all of the duodenal samples   The average of the tryptic activity in the half hour duodenal samples of the 22 cases of pernicious anemia, before activation with enterokinase, was 15 2 mgm per cc and 21 5 mgm per cc following the activation   The value obtained before activation was approximately the same as that found in normal persons, but the value after activation was distinctly lower   There was an increase of only 49 per cent of the tryptic value after activation with the enterokinase, as compared with the increase of 83 per cent found in the normal specimens   Therefore, it appears that there is a decreased secretion of trypsinogen in these patients, although their values for tryptic activity by the usual tests fall within the normal range   It is evident from these observations that there is a normal amount of enterokinase secreted by the duodenum   The average of the lipolytic activity in the half hour samples of the cases of pernicious anemia was slightly lower than the normal values   The average amylolytic values were approximately equal to those found in the normal persons

The 22 cases of pernicious anemia were grouped in Table V and in Chart 2 according to their clinical and hematological status at the time of the analyses   Those patients having moderate to advanced central nervous system involvement usually had a much lower tryptic activity in the duodenal contents, not only when the tryptic values were compared with the normal findings, but even when these values were compared with those obtained in the patients with pernicious anemia having early or no

TABLE IV

The volume, color, pH, trypsin before and after activation with enterokinase, lipase, and amylase in the fasting gastric juice and in the duodenal contents following acid stimulation in 22 cases of pernicious aremia

Case number	Date	Specimen	Volume	Color	pH	Tryp sin	Tryp sin and tryp-sin ogen	Lip ase	Amyl ase
	1932		cc			mgm per cc	mgm per cc	cc N/10 NaOH	mgm glu cose
1	July 6	Fasting gastric							
		1st half hour	45	Green		12 4	16 4	1 0	0 9
		2d half hour	17	Yellow					0 7
2	July 11	Fasting gastric	3	Negative	8 4	1 9	1 7	0 2	0 8
		1st half hour	56		6 8	3 3	4 6	0 2	0 3
		2d half hour	13		8 4	8 0	11 0	1 1	0 9
	October 12	Fasting gastric	13	Bile	8 2	0 2	1 0	0 0	1 1
		1st half hour	52	Amber	8 4	8 2	10 8	0 8	0 9
3	July 15	Fasting gastric	40	Negative		3 7	4 2	0 2	1 7
		1st half hour	56			9 3	11 5	0 5	1 4
		2d half hour	53			16 1	25 3	1 2	1 8
4	July 22	Fasting gastric	13	Negative		0 2	0 2	0 2	0 6
		1st half hour	36	Yellow		9 9	17 2	2 2	0 7
5	July 27	Fasting gastric	18	Negative		0 0	0 3	0 1	2 3
		1st half hour	150	Green		20 0	29 1	1 0	0 8
		2d half hour	63	Green		19 5	34 8	1 0	1 5
6	August 11	Fasting gastric	45	Yellow		2 0	2 6		0 8
		1st half hour	79	Yellow		17 2	24 6		0 8
		2d half hour	50				30 8		1 1
7	August 30	Fasting gastric	3	Negative		0 5	0 5	0 1	0 9
		1st half hour	120	Yellow		9 2	15 2	0 7	2 2
		2d half hour	58	Yellow		12 3	16 2	1 2	2 5
8	September 1	Fasting gastric	24	Negative	8 7	0 0	0 0	0 0	1 3
		1st half hour	75	Yellow	7 6	29 7	35 2	3 1	0 9
		2d half hour	8	Yellow	7 6	24 8	38 2	3 2	3 0
9	September 7	Fasting gastric	13	Negative	8 4	0 6	0 7	0 0	0 9
		1st half hour	44	Brown	7 6	13 5	20 0	3 2	0 8
		2d half hour	50	Brown	7 8	14 2	28 3	4 4	2 1
10	September 9	Fasting gastric							
		1st half hour	88	Amber	8 0	15 2	26 6	1 7	2 5
		2d half hour	10	Brown	8 6	16 3	21 2	1 7	3 0

TABLE IV—*Continued*

Case num ber	Date	Specimen	Vol ume	Color	pH	Tryp sin	Tryp- sin and tryp- sino- gen	Lip ase	Amyl ase
	1932		cc			mgm per cc	mgm per cc	cc N/10 NaOH	mgm glu cose
11	September 13	Fasting gastric	16	Bile	7 8	2 7	5 3	0 9	2 9
		1st half hour	110	Amber	7 8	12 4	26 2	4 2	3 4
		2d half hour	45	Yellow	7 8	12 4	21 4	4 7	4 8
12	September 14	Fasting gastric	10	Negative	8 2	0 2	1 0	0 1	2 2
		1st half hour	95	Brown	7 8	18 8	27 2	3 4	1 9
		2d half hour	25	Brown	8 2	16 7	24 4	3 0	2 3
13	September 16	Fasting gastric	2			0 0			
		1st half hour	32	Yellow	7 8	20 8	28 0	4 0	1 1
14	September 19	Fasting gastric	41	Negative	8 2	0 4	0 5	0 2	2 5
		1st half hour	63	Brown	8 2	15 4	17 4	2 4	2 1
		2d half hour	50	Yellow	7 8	10 4	14 8	2 6	2 3
15	September 20	Fasting gastric	32	Negative	8 6	0 4	1 0	0 0	2 3
		1st half hour	30	Brown	7 8	17 2	26 0	3 4	0 8
		2d half hour	19	Yellow	8 2	15 4	22 5	3 0	1 8
16	September 21	Fasting gastric	58	Bile		2 6	5 5	0 8	3 1
		1st half hour	31	Yellow		9 5	18 2	2 2	1 5
		2d half hour	21	Blood		23 6	29 2	3 2	3 8
17	September 26	Fasting gastric	14	Bile	7 8	12 8	12 2	0 6	5 2
		1st half hour	80	Amber	7 6	38 2	34 8	3 0	2 9
		2d half hour	46	Amber	8 2	20 0	25 0	1 7	3 7
18	September 28	Fasting gastric	5	Bile	8 0	1 5			
		1st half hour	65	Amber	7 8	10 2	16 9	1 0	2 7
		2d half hour	11	Blood	8 2	8 9	13 2	1 3	2 6
19	September 29	Fasting gastric	9	Negative	8 6	0 6	1 0	0 2	3 4
		1st half hour	65	Amber	8 2	10 0	15 6	1 2	1 9
20	October 3	Fasting gastric	29	Negative	7 8	2 7	6 0	0 3	2 3
		1st half hour	129	Amber	8 0	17 4	26 6	3 0	2 3
		2d half hour	46	Amber	8 2	17 2	22 0	2 0	3 7
21	October 5	Fasting gastric	3			0 4			
		1st half hour	82	Amber	8 5	9 2	12 0	1 2	0 2
		2d half hour	50	Amber	8 4	12 8	16 0	1 2	0 2
22	October 17	Fasting gastric							
		1st half hour	22	Amber	8 6	12 8	16 9	1 8	0 9
		2d half hour	6	Amber	8 4	13 8	20 4	2 6	1 3

TABLE V

Mean values with probable error of the mean of the trypsin, trypsin plus trypsinogen, lipase, and amylase of half hour samples in the various groups of patients with pernicious anemia and in normal subjects

	Number of cases	Mean values			
		Trypsin	Trypsin and trypsinogen	Lipase	Amylase
		mgm per cc	*mgm per cc*	*cc N/10 NaOH*	*mgm glucose*
I Normals	5	15 4±0 67*	27 2±1 62	3 2±0 12	1 5±0 14
II Pernicious anemia					
1 Cases having moderate to advanced central nervous system involvement	14	12 2±0 63	18 0±0 94	2 1±0 23	1 6±0 18
2 Cases having early or no demonstrable central nervous system involvement	8	20 1±1 18	27 8±1 08	2 4±0 17	2 2±0 18
3 All cases having RBC below 3 0 million per c mm	5	12 6±0 75	17 8±1 03	1 4±0 14	1 1±0 17
4 All cases having normal RBC	17	15 7±0 95	21 8±1 13	2 4±0 19	2 0±0 16
5 Cases having moderate to advanced central nervous system involvement and RBC below 3 0 million per c mm	4	11 8±0 93	16 2±0 56	1 4±0 16	1 0±0 21
6 Cases having moderate to advanced central nervous system involvement and a normal RBC	10	12 3±0 86	18 8±1 24	2 3±0 29	1 7±0 22
7 Cases having early central nervous system involvement and RBC below 3 0 million per c mm	1	17 2	24 6		1 0
8 Cases having early or no demonstrable central nervous system involvement and normal RBC	7	20 2±1 31	27 8±1 21	2 4±0 18	2 3±0 19
9 Cases able to maintain RBC at normal levels on liver extract derived from 300 or less grams of liver	6	15 4±1 61	21 8±2 04	2 1±0 31	1 6±0 16
10 Cases requiring more than the amount of liver extract derived from 300 grams of liver	9	14 6±1 05	22 4±1 52	2 4±0 28	2 2±0 23
11 All cases	22	15 1±0 79	21 6±0 95	2 2±0 16	1 8±0 14

* Probable error of mean
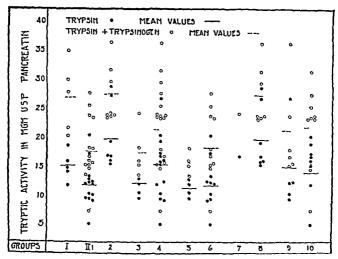
CHART 2   INDIVIDUAL VALUES OF TRYPSIN AND OF TRYPSIN AND TRYPSINOGEN
PLOTTED ACCORDING TO GROUPING IN TABLE V

demonstrable central nervous system involvement   This finding was
more evident when the tryptic activity of the samples after activation
with enterokinase was studied   The amylolytic and lipolytic activity of
the duodenal contents of the patients having moderate to advanced cen-
tral nervous system involvement was also slightly decreased, but not to
such a degree as the tryptic activity   One patient (Case number 2) with
marked central nervous system involvement had the lowest trypsin values
in the series, and, repeating the test three months later, similar results
were obtained   Of the four patients having moderate to advanced central
nervous system involvement, who had normal amounts of tryptic activity
in the duodenal contents three had recently shown definite improvement
of symptoms referable to involvement of the central nervous system while
receiving liver extract by injection

   There were only five patients having red blood counts below 3 0
million per c mm , but in all there was a definite decrease in lipase and
amylase and a slight decrease in trypsin   Fifteen of the patients had
been studied by this department for a sufficient length of time to be used
for determining the relationship of the duodenal contents with the main
tenance dose of liver extract   The averages of the tryptic and lipolytic
activity in those patients who were able to maintain the blood at normal
levels by taking the amount of liver extract derived from   or

less of whole liver daily, were approximately equal to those found in the patients requiring more liver extract   The average of the amylolytic activity of the latter group was slightly higher than that of the former   The one patient who was able to maintain the blood at normal levels while taking very small amounts of liver extract, however, had the highest tryptic activity in the duodenal contents of any of the fifteen patients

DISCUSSION

In the 22 cases of pernicious anemia studied, there were no cases in which there was an absence of the pancreatic enzymes in the duodenal contents   This is in agreement with the findings of Crohn (7) (8), Chace and Myers (9), White (10), and McClure and associates (11), but is at variance with the findings of Cheney and Niemand (21)   However, Cheney and Niemand based their opinion on the analyses of the fasting gastric contents   From our work it is evident that fasting gastric juice findings are not a satisfactory index of the external secretory function of the pancreas

Although there were no cases in which there was a total absence of the pancreatic enzymes, it is interesting to note that, in all the patients with pernicious anemia having decreased amounts of tryptic enzymes, there was moderate to advanced central nervous system involvement present We feel that this finding of low enzymatic activity in the duodenal contents of cases with achylia gastrica is of more significance than similar findings in cases having normal gastric function, because there was no free hydrochloric acid or pepsin to inhibit the pancreatic enzymes and, in addition, there was only a small amount of gastric juice in such cases to dilute the duodenal contents   The finding of decreased tryptic activity in the patients with pernicious anemia having moderate to advanced central nervous system involvement, suggests the possibility that a decrease in the external secretory function of the pancreas might be of etiological importance in the production of central nervous system involvement in pernicious anemia   A study of the duodenal contents of a larger series of cases is necessary to verify this possibility

In this limited series, Case number 8 is the only one suggesting any correlation between maintenance dose of liver extract and the amount of pancreatic enzymes in the duodenal contents   This patient had one of the highest tryptic values in the whole series and was able to maintain the blood at normal levels for a period of $2\frac{1}{2}$ years while taking the amount of liver extract derived from 100 grams of whole liver every other day   This suggests that the maintenance dose of liver extract in some patients with pernicious anemia may be influenced by the degree of activity of the pancreas, however, the findings in the remaining patients show no such relationship   The probability still remains that in the majority of the patients receiving an adequate diet and having no complications, the

maintenance dose of liver extract is governed by the ability of the patient to absorb the active principle from the gastro intestinal tract, as suggested by Castle and his associates (26)

CONCLUSIONS

1 Twenty two cases of pernicious anemia were studied and all showed pancreatic enzymes in the duodenal contents

2 The determination of pancreatic enzymes in the fasting gastric contents is of no value in estimating pancreatic activity

3 The incubation of duodenal contents with enterokinase is necessary in order to determine the total amount of proteolytic enzymes secreted by the pancreas

4 The ability of the duodenal mucosa to secrete enterokinase is apparently not impaired in pernicious anemia

5 All patients with pernicious anemia who had decreased tryptic activity showed moderate to advanced central nervous system involvement

6 With the possible exception of one case, there was no correlation between maintenance dose of liver extract and the activity of the pancreatic enzymes

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HEAT CRAMPS   A CLINICAL AND CHEMICAL STUDY

By JOHN H TALBOTT AND JOST MICHELSEN

(*From the Fatigue Laboratory, Morgan Hall Harvard University Boston and the Six Companies Hospital Boulder City Nevada*)

The regulation of the interchange of water and salt in the body is a delicate and very precise mechanism   Haldane and Priestley (1) found no change in the percentage of hemoglobin in the blood after ingestion of large amounts of water plus salt   Later Priestley (2) reported a diminu tion in the electrical conductivity of the serum following an increased water intake and an increase of conductivity after ingestion of salt solu tion   Crawford (3), Adolph (4) and others confirm these findings Because of experimental difficulties fewer data are available concerning a restricted intake of these constituents in normal men   The increased output of water and salt, however, especially in urine, sweat and vomitus, has been studied extensively   Gamble and Ross (5) found a marked reduction in body weight in dogs after experimental pyloric obstruction This loss of weight and inability to assimilate ingesta from vomiting was essentially referable to a loss of water plus sodium and chloride ions Sweating is another mechanism by which salt and water are lost from the body   In a temperate climate this salt loss is small, even in heavy work, and is not responsible for a fall of the salt level of the blood serum (6) In a hot climate, however, sweating may reach a magnitude sufficient to alter various constituents of the blood during a work period of several hours duration (7)   If this changed composition of blood reaches suffi cient magnitude, then a break down of some function of the living organ ism may be expected

Clinically the failure of the human organism to cope with a high environmental temperature is generally associated with the conditions known as sun stroke, heat stroke and heat exhaustion   There is another malady less well known, but nevertheless definite in its characteristics, that is frequently seen in workers in extreme heat   This is known as heat cramps   In 1904 Edsall (8) described two cases of painful cramps admitted to the hospital and later (9) attributed them to exposure to intense heat   Miners and firemen frequently report off duty because of severe muscle cramps when working in high temperatures   This malady has been adequately described and satisfactorily named but the cause has not been clearly defined   This communication is a study of several cases of heat cramps with a simple hypothesis for their origin

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Clinically the failure of the human organism to cope with a high environmental temperature is generally associated with the conditions known as sun stroke. heat stroke and heat exhaustion   There is another

During the summer of 1932 members of this laboratory were investigating the physiological and pathological effects of high temperature upon the living organism Boulder City, Nevada, as the center of operations for the construction of Hoover Dam, offered unusual opportunities for this study This city, only seven miles from the site of Hoover Dam, is situated in the Colorado River Basin Desert There were approximately 2500 men working on the Dam who lived in Boulder City during the recent summer [1] Figure 1 gives the daily maximum temperatures in
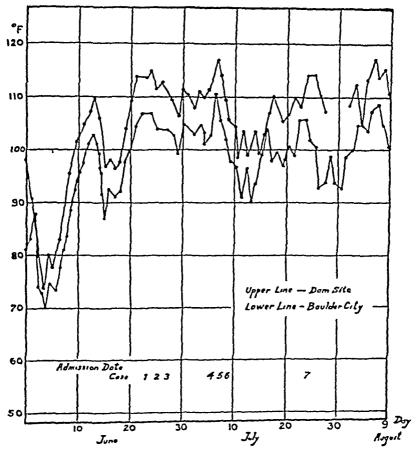
FIG 1   MAXIMUM DAILY TEMPERATURE OBSERVATIONS DURING THE SUMMER OF 1932

Boulder City and at the Dam site during this period The admission dates for the cases discussed are noted on this figure There were roughly four hot spells of 4 to 5 days each, and during these periods of heat all of

[1] The employees of the Six Companies Construction Company were cared for at their own Hospital This is a modern 60-bed hospital, well equipped to care for medical and surgical cases, located in Boulder City The authors were given a "free hand" in the diagnosis, treatment and investigation of all suspected and verified cases suffering from the ill effects of heat.

the cases were admitted to the hospital   All of the suspected heat cases were seen within 15 minutes after admission and within 45 minutes after leaving the Dam   No known medication was given before they were seen by us   Forty cc of venous blood was drawn shortly after we saw the patient, and following this a history was taken and physical examination was done   Thus the significant observations were completed before hypodermic, subcutaneous or intravenous therapy was begun

The hospital cases admitted because of the ill effects of heat were few in number when a comparison is made with the preceding summer   While accurate statistics are not available concerning the mild and moderate cases suffering from heat, there are 17 cases in the insurance records in which death was attributed to heat from May to October, 1931   There were no deaths from heat in the summer of 1932, and according to sources considered to be reliable the number of mild and moderate heat cases was markedly below that for the summer of 1931   Greatly improved living quarters, milder temperature, abundance of cooled drinking water and acclimatization were probable factors in this reduction

There are seven cases described in this paper   Five of these were suffering from heat cramps and two from heat exhaustion   All of the cases of heat cramps admitted to the hospital during our stay in Boulder City are included

METHODS

The methods employed for the determination of all of the urinary constituents and the majority of the blood constituents are given in a previous paper (6)   The chlorides of the serum and cells were determined according to Wilson and Ball (10)   The micro method of Folin and Malmros (11) was used for blood sugar   The total fixed base of the serum was determined after Van Slyke et al (12)   The morphology of the blood was studied in the accepted clinical fashion

Heparin was added as an anticoagulant to the drawn blood   Blood for estimation of lactic acid sugar and formed elements was removed at once The remainder, about 35 cc , was equilibrated at 37 5° in a water bath at the approximate value $Pco_2 = 40$ mm Hg   After 15 minutes equilibration, blood for measurements of oxygen capacity and CO_2 content was removed   The remaining whole blood was then centrifuged for three 20 minute periods   Calculation of cell volume at infinite time was then possible according to Hirota's method (13)   The various serum determinations were performed on the separated plasma and the cell water and cell chloride on the separated cells

RESULTS

In no instance was arterial blood secured and the equilibration of venous blood was carried out under tensions of carbon dioxide that varied somewhat in the series   Thus Table I of experimental observations is supplemented by Table II, which permits a ready and more exact comparison of the acid base equilibrium in the serum   The original data may be recalculated assuming a constant value for one of the several

30

TABLE I

Experimental observations on oxygenated blood

Case number	Date and time (1933)	Oxygen capacity (m Eq per liter of blood)	P_{CO_2} (mm Hg)	Total $(CO_2)_b$ (m Eq per liter of blood)	Total $(CO_2)_s$ (m Eq per liter of serum)	Cell volume (per cent)	$(Cl)_s$ (m Eq per liter of serum)	$(Cl)_c$ (m Eq per liter of cells)	$(H_2O)_c$ (cc per liter of cells)	(Total fixed base)$_s$ (m Eq per liter of serum)	$(HPO_4)+(H_2PO_4)_s$ (m Eq per liter of serum)	(Protein)$_s$ (grams per liter of serum)	(Calcium)$_s$ (m Eq per liter of serum)	(Lactic acid)$_b$ (m Eq per liter of blood)	(Sugar)$_b$ (mgm per 100 cc of blood)	Red blood cells (millions)	White blood cells
1	June 21 12 00 M	10 73	36 8	18 68	22 59	52 0	92 0	47 7	702	144 5	2 7	93 8	5 2	1 5		6 18	15,150
	June 25 1 15 PM	8 96	39 1	20 31	21 73	43 5	99 6			146 5		68 0			107	4 96	13,100
	June 26 7 45 AM	8 71	42 7	21 38	26 80	43 2	99 9		713	145 5	2 6	72 8	5 3	1 5	112	4 79	9,250
	July 16 5 30 AM	8 86	45 2	19 19	24 83	43 8	108 0	52 9	712	148 0	2 2	70 2		1 5	94	4 90	5,100
	June 25 1 00 PM	8 30	36 8	19 70	21 83	41 9	109 0					68 8			116		9,300
2	June 27 10 00 AM	8 10	35 7	20 52	24 42	42 6	107 8	51 6	711	151 5	2 4	67 2	5 1	1 1	109	5 12	9,250
3	July 6 11 30 AM	8 30	32 5	22 78	26 08	43 3	99 2	47 5	716	150 9	1 7	70 9	5 1	1 8	111	4 41	6,050
4	July 8 2 00 AM	9 14	45 9	19 11	25 56	49 6	99 0	51 6	722	150 5	2 4	75 6	5 2		106	4 93	7,900
5	July 9 9 00 AM		35 9	19 62	23 36	49 9	101 6	53 0	728		2 2	75 2				5 20	
	July 16 4 00 PM						102 0					76 3				4 27	
6	July 9 11 00 AM	8 34	37 1	16 20	18 61	45 4	96 4	54 1	738	148 3	2 5	64 0		1 5	95	4 50	8,000
	July 10 8 00 AM		35 4	16 80	19 04	43 5	100 4	59 8	733	147 0	1 6	72 4	5 1	1 7		4 60	12,600
	July 11 9 00 AM	7 75	35 7	19 96	21 73	41 7	104 0	57 6	723	147 2	2 2	67 8	5 1	1 5			
7	July 11 5 00 PM	7 62	42 3	19 89	29 11	40 7	105 0	50 0	721	152 2	3 5	69 5		2 9	99	5 10	4,800
	July 23 5 30 PM	9 12										83 2	6 5		107	5 24	10,500
	July 21 9 00 PM	8 64	42 0	18 72	23 08	47 5	94 8		720	147 5	2 3	73 1	5 5		98		6,700

variables $pH_s = 7\,45$ has been arbitrarily selected in the construction of Table II This treatment has certain disadvantages, nevertheless its use is probably justified

TABLE II

Electrolytes of true plasma and r_{Cl} at $pH_s = 7\,45$
(*Concentrations are expressed in m Eq per liter of serum excepting r_{Cl}*)

Case number	Date	$(HCO_3)^-$	$(Lactate)^-$	$(Cl)^-$	$(HPO_4)^{--}$ $+(H_2PO_4)^-$	$(Pro-teinate)^-$	Σ Anions	Total base	r_{Cl}
	1938								
1	June 24	21 12	1 5	92 7	2 69	23 1	141 1	146 1	65
	June 26	25 10	1 5	100 5	2 58	17 9	147 6	146 3	
	July 16	22 98	1 5	109 4	2 18	17 3	153 3	148 8	62
3	June 27	23 13	1 1	108 2	2 44	16 5	151 4	151 8	62
4	July 6	25 27	1 8	98 7	1 72	17 5	145 0	150 6	62
5	July 8	23 52	1 5	99 0	2 39	18 6	145 0	151 5	66
	July 9	21 52	1 5	102 3	2 17	18 8	146 3		65
6	July 9	16 52	1 5	98 4	2 55	15 8	134 8	149 6	66
	July 11	22 70	1 7	105 6	1 61	16 7	148 3	148 1	71
	July 14	21 38	1 5	106 5	2 18	17 1	148 7	148 5	68
7	July 23	21 18	2 9	96 1	3 55	20 5	144 3	153 2	65

It is possible to calculate very accurately the total carbon dioxide of whole blood at $pH_s = 7\,45$ with knowledge of the oxygen capacity and the partial pressure of CO_2 during equilibration (14)   The spread between total CO_2 of whole blood and true plasma was assumed to be the same for a given specimen of blood at the observed pH_s and at $pH_s = 7\,45$ Total CO_2 of true plasma at $pH_s = 7\,45$ was calculated on this basis

Values for pH are derived from the equation

$$pH_s = pK_s + \log (BHCO_3)_s - \log (H_2CO_3)_s,$$

where

$$pK_s = 6\,10,$$

$$m\ Eq\ (H_2CO_3)_s = 0\,031\ (H_2O)_s\ Pco,$$

and

$$(BHCO_3)_s = (\text{total } CO_2)_s - (H\ CO_3)_s$$

Base bound by protein is calculated according to Van Slyke, Hastings, Hiller and Sendroy (15)

$$m\ Eq\ (BP)_s = 0\,104\ (P)_s\ (pH_s - 5\,08),$$

where

$$(P)_s = \text{grams of protein per liter of serum}$$

and

$$pH_s = 7\,45$$

Values for serum volume and serum and cell chloride were corrected to $pH_s = 7\,45$ by reference to unpublished observations on dog s blood

The following formulae were employed

$$V_{c\ cor} = V_{c\ obs} + 4\ 0(pH_{s\ obs} - 7\ 45),$$

$$Cl_{s\ cor} = Cl_{s\ obs} - 13\ 3(pH_{s\ obs} - 7\ 45),$$

$$Cl_{c\ cor} = Cl_{c\ obs} + 20\ 0(pH_{s\ obs} - 7\ 45)$$

The shift in serum volume and chloride for dog's blood within the physiological range is probably close enough to that of man to introduce no significant error in its use   Lactate of serum is calculated from its concentration in whole blood, assuming the concentration in cells and serum to be equal

The expression Σ anions represents the corrected sum of the anions, which, if all anions were accounted for, should be approximately equal to the corresponding value for total base   Inspection of these values shows a striking difference between the blood on admission and after recovery A marked discrepancy between total anions and cations is found only in the admission blood   Values for $(HCO_3)^-$, except in Case 6, are only slightly below an average normal of 24 m Eq   The lactate and inorganic phosphate of the serum are only slightly elevated, except in Case 7, where both values are about twice the normal ones   The proteinate ions are considerably increased because of a concentration of the blood proteins related to the degree of dehydration   Shifts of these four electrolytes of this magnitude and direction are frequently seen in a physiological state no more abnormal than that accompanying moderate exercise   It is our impression that the distribution of the chloride ion is regulated by a mechanism more resistant to change, and that an elevation or depletion of the blood chloride is seen only in disease   Thus the low values for chlorides seem to us most significant of all

In all cases the blood taken on the second day of hospitalization after treatment had begun shows chloride values only slightly below normal This significant rise in serum chloride coincided clinically with relief from cramps   Cases 2 and 3, who did not complain of cramps, had normal chloride values   The total base of the serum was low on admission and in Cases 1 and 6 did not reach the normal value of 152 m Eq during recovery

The concentration of red blood corpuscles corresponds closely to the oxygen capacity and cell volume   Calculation of the cell hemoglobin gives an average value of 19 6 m Eq per liter of cells   This is slightly below the value of 20 3 given by Dill (16) for 7 normal subjects   There was some tendency for the white cells of the blood to be elevated with only slight increases in the polymorphonuclear percentage   All of the patients with elevated white counts had normal rectal temperatures

Table III contains data concerning water balance   The fluid intake, high as it may seem, is probably considerably below the average daily intake for workmen during the hot periods   The values for salt intake

TABLE III

Daily observations on fluid intake and output

Case number	Date	Comment	Milk	Fluid parenterally	Total fluid intake	Sodium chloride intake (approximate)	Urine	Specific gravity	Sodium chloride	Total nitrogen	Creatin	Creatinine	Inorganic phosphate
			cc	*cc*	*cc*	*grams*	*cc*		*grams*	*grams*	*grams*	*grams*	*grams*
	1958												
1	June 24	18 hours	1300	3000	6000	31	825	1022	0 10	14 45	0 93	0 94	1 06
	June 25		3700		5150	15	3210	1007	3 92	24 75			
	June 26		2000		5400	8	1800	1010	7 34	22 82	0 00	0 00	0 99
	June 27	Home	1500		5100		1000	1024	4 48	14 16	1 16	1 42	2 14
	June 28		900		6600		1190	1028	12 90	15 65	0 79	1 10	1 49
	June 29		600		4400		885	1018	14 59	7 89	0 40	0 65	0 69
	June 30	At work	600		3400		1290	1023	20 00	10 72	0 89	1 07	1 20
	July 1		400		3900		850	1027	10 12	8 48	0 76	0 94	1 06
	July 2		400		7200		570	1031	6 56	6 47	0 51	0 63	0 91
	July 16		1100		4800		875	1028	7 09	10 00	0 70	1 08	1 21
2	June 25	24 hours	1500	1000	6500	32	720	1026	8 42				
3	June 27	18 hours	2000	3000	4700	17	2530	1008	21 10				
4	July 6	24 hours	2000	1000	5500	17	740	1004	2 19				
5	July 8	30 hours	2000	1000	4500	8	1740	1012	5 33				
6	July 9	Home	1200		7500		770	1013	2 59	3 59	0 00	0 00	1 70
	July 10	20 hours	3000	1000	4400	23	775	1018	3 25	6 86	0 34	0 57	0 79
	July 16		2200		3500	9	1980	1003	15 86	10 16			
7	July 9		2500		6600		3400	1011	4 80	7 80			
	July 10		2400				2030	1008	8 53	14 30			
	July 11	Soft diet	1000				1700	1024	11 48	24 51			
	July 14	At work					1295	1021	9 84	23 50			
	July 23	12 hours				13	710	1024	3 07	11 17	0 07	0 47	0 48
	July 24	Home		1000	3000		575		3 90	10 77	0 56	0 97	1 24

are only approximate but show in a striking degree the retention of salt [2] by the body early in recovery   This was most marked in Case 1 where the intake of 45 grams of salt in 42 hours was accompanied by a urinary excretion of only 4 grams   Only a small amount of the salt not accounted for was lost in the stools and by sweating, the remainder was retained by the body   Values for total nitrogen in the urine are high for the first two days of observation   In an unpublished study of the healthy workmen at the Dam no such increase was found in any of the 24-hour urine specimens   It seems unlikely that the high nitrogen excretion can be attributed to exercise, to the high environmental temperature or to elevated body temperature   Increased destruction of muscle protein accompanying the cramps may be the source of some of the additional nitrogen [3]   The utilization of tissue protein for fuel is probably responsible for most of the increase

CLINICAL DISCUSSION

The characteristics that distinguish heat cramps from other varieties of cramps are few   The past history may reveal a previous attack of cramps while working in a high temperature   J C (Case 7) was the only patient in our series that admitted having had heat cramps previously   Individual variation in susceptibility is undoubtedly important   This response is intimately related to the phenomenon of acclimatization to the heat   Adequate proof of acclimatization is not at present available but certain considerations strongly suggest this   Acclimatization seems to be complete after the 3rd to 5th day   It is our belief that any given individual is more resistant to cramps after this 5-day period, other factors being equal   Moss (17) noted that colliers acclimatized to a high temperature lost twice as much sweat under the same conditions as unacclimatized subjects   Our observations (7) do not agree with these findings

There may be a prodromal period of 1 to 3 days before the onset of acute symptoms   During this time the salt intake may be exceeded by the salt excretion   An alcoholic bout with diminished intake of food and salt may be followed by sufficient gastric irritation and vomiting to lower appreciably the salt level of the body   In Case 1 the 3-day prodromal period was preceded by an alcoholic bout, the severity and importance of which could not be ascertained   Vomiting with loss of electrolytes preceded the muscle cramps in Cases 1, 5 and 6, and nausea was a symptom in Case 7 as well   Diarrhea may be a symptom but was not noted in

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[2] In most instances the use of the word salt implies sodium chloride, as based on chloride determinations

[3] This process is similar to that observed by Morawitz in cases of non-nephritic uremia with hypochloremia   Morawitz, P , and Schloss, M , Klin Wchnschr , 1932, xi, 1628   'Extrarenale' Albuminurie und Urämie

any of our cases of cramps   There was a prevailing impression that profuse and explosive diarrhea was an accompaniment of most of the severe heat cases in the summer of 1931   No figures are available showing the incidence of diarrhea in uncomplicated heat cramps and in other ill effects of heat   It is interesting to note that cases of cholera as described in the literature have muscle cramps associated with prolonged diarrhea   As in heat cramps the vomiting and diarrhea may seriously deplete the salt in the body

The presenting symptom is generally pain in various groups of muscles which prevents the subject from continuing work   Tingling in the fingers and toes may precede or accompany the cramps   Edsall (9) observed extreme irritability and fibrillary twitchings of the involved muscles in his patients with heat cramps   Deep or superficial palpation produced pain   In contrast in none of our cases were there fibrillary twitchings nor did palpation produce pain   However, active motion of the affected muscles was painful in our most severe cases

The predilection of certain groups of smooth and striated muscles for cramps is worth noting   The muscles of the calf and the flexors of the forearms were the commonest sites   The muscles of the abdominal wall were also affected in some instances quite apart from the smooth muscle of the gut   It is our belief that the vomiting and diarrhea may be referable to irritation of the smooth muscle by a process similar to that causing cramps in striated muscle   The effect of diminished blood chloride upon the heart muscle is an interesting problem for speculation   Dr D B Dill suggested to us that the symptoms of Case 4 might be referred to such salt depletion   This patient came into the hospital with symptoms in some respects resembling those of coronary occlusion   His past cardiac history was not important   The physical examination was negative   His serum chlorides were below normal and he responded in a typical fashion to intravenous saline   The retention of salt during the following 24 hours is further proof of the lowered salt level of his body   The hospital does not have an electrocardiograph and no record was taken

The relation of heat cramps to heat exhaustion or heat stroke has not been clearly defined   In common with heat stroke or exhaustion is the exposure to a high temperature and the resultant loss of large volumes of sweat   Haldane (18) found a maximum loss of 5½ pounds per hour in seasoned colliers at work, and a much smaller loss of sweat in unacclimatized workers   That the amount of sweating is a function of external temperature rather than duration of exposure was substantiated by experiments of Hunt (19)   In a Turkish bath he found that the sweat loss was about 2 pounds per hour when the temperature was between 60° and 80° C   Sweating was as free at the end of 3 hours as it was early in the experiment   This agrees with observations on ourselves during

the summer   The comparison of heat stroke with cramps and with prostration reveals less similarity beyond this point   Clinical evidence against a common causal agent in heat stroke and heat cramps is afforded by the observation made by Willcox (20)   He noted that the convulsions in heat stroke were made worse after intravenous saline injections   The opposite effect might be expected if the essential pathological process were a depletion of salt and water in the central nervous system   Cramp cases show only a slight elevation of body temperature   None of the cases in our series had a temperature above 101° F   after admission to the hospital   It is conceivable, but unlikely, that the temperature was higher before admission   In 37 cases reported by Gauss (21) temperatures were subnormal in most of the subjects and were not elevated in any   Cases of heat exhaustion similarly have only a slightly elevated temperature   In contrast the heat stroke or heat hyperpyrexia patients run a fever frequently as high as 110° F   with a correspondingly high pulse rate (Willcox, 20)   The heart rate was as intimately related to body temperature, other factors being equal, in these cases as it had previously been found to be in hot-room experiments (22)   In none of our cramp cases was the pulse over 90   Restlessness and delirium were noted in one case only, Case 4, and he regained his mental equilibrium soon after admission to the hospital   In view of the number of cases diagnosed as sun stroke in the cities during the summer months, it is worth noting the absence of cases at Boulder City during the summer of 1932   Many of the men at work scaling the cliffs were exposed to the sun's rays for several hours each day   The shade temperature was frequently 45° and the temperature of the rocks was 55° to 60° C   Each day between the hours of 10 and 12 in the morning one of us (J H T) regularly played three hard sets of tennis in the direct sun without any head covering   The question of whether a healthy individual, accustomed to the heat for a few days, could succumb to sun stroke is an important one and not fully answered by the cases in the literature to date

An important criterion in substantiating a diagnosis of heat cramps is the marked relief following parenteral injection of large amounts of normal salt solution   All of our cases in this series were free from symptoms within six hours after starting treatment

In summary, then, a diagnosis of heat cramps may be made with the following conditions satisfied   (1) Exposure to a high temperature at work   (2) Rapid loss of salt in the sweat, that is not replaced   (3) Painful muscle cramps   (4) Diminished concentration of chloride and base in the blood and likewise in the body tissues   (5) Rapid amelioration of symptoms after therapy

PATHOGENESIS

The pathogenesis of heat cramps has been variously assigned according to the period in which the several theories were advanced   In 1904

Edsall (8) proposed, as the pathological process, acute degeneration in the muscle Some time later an infectious agent was considered but this was never sufficiently proved In recent years interest in this subject has been transferred to pathological physiology In 1923 Haldane in his discussion of a paper by Moss (23) stated that he believed miners' cramps could be attributed to water poisoning Haldane further says, "When a man is working his blood is shunted away from the kidneys and excretion of urine stops If the kidneys were working normally they would excrete the excess of water and save the man from cramps" In consideration of our quantitative observations it is desirable to define water poisoning and to qualify the assertions concerning the excretion of urine

The body tissues of patients with heat cramps contain hypotonic fluid as seen from Table II, but this is not from the excess of water, rather it is from the depletion of total base and chlorides Likewise after the beginning of treatment, if salt and water are retained to make up the deficit, a gain in weight during recovery would be expected If the fundamental process at fault were an increase of total body water, then the patients after the beginning of treatment would lose weight because of the diuretic action of salt and water The facts observed in our cases that were weighed on admission and at discharge are as follows Case 7 gained 3 0 pounds in the first 24 hours and Case 6 gained 7 0 pounds in 48 hours In regard to kidney function it is our belief that the principal reason for low kidney excretion during working hours in a hot climate is peripheral dilatation of the capillaries for dissipation of heat, rather than a primary shut down of the kidneys Heller and Smirk (24) studying rabbits and rats demonstrated a water diuresis (diuresis following an elevated fluid intake) in the presence of depleted water reserves There is no evidence that under the above conditions for man the kidneys are not capable of excreting any excess of water that may be taken in

Haldane (23) predicted a lowering of the blood chlorides of 3 to 4 per cent as a possible accompaniment of heat cramps In this series the mildest case showed a 2 per cent reduction in serum chlorides and the severest case a 10 per cent reduction below the accepted normal minimal value Direct evidence is not at hand showing that the drop in the chloride level of the blood is followed by a similar depletion of the chloride in the intercellular spaces It is generally assumed that the concentration of electrolytes in the serum and intercellular spaces is approximately equal If these are the facts then a loss of water and salt from the blood is followed by a partial replacement from the tissues In the condition under discussion there is a loss of salt and water from the body with a concomitant replacement of water only If this major process is continued, irrespective of the secondary processes, there will eventually be a lowering of the chloride level below the normal range It is our belief that when a critical level for the chlorides is reached in working individuals

muscle cramps will occur   It seems likely that the critical chloride level is a function of individual susceptibility, acclimatization and the length of the prodromal period

TREATMENT AND PREVENTION

If the cause of heat cramps is essentially a loss of salt and water from the body tissues then treatment should provide for restoration   The most rapid replacement of salt and water is by means of intravenous saline solution   In our study only sodium chloride was used   A larger number of cases would have enabled us to use other salts in treatment   A control study with glucose infusions without salt would likewise have been desirable   In addition to the saline infusions an exclusive milk diet was given during the first 24 hours   This simple regime was highly satisfactory in all of the cases

It is possible to prevent cramps by providing a daily supply of salt greater than that lost in the sweat   This amount may be determined by knowing the approximate amount of chloride excreted in a 24-hour urine specimen   In our experience less than 3 grams of salt per day in the urine does not provide for a satisfactory margin of safety   A high salt diet may be provided in a number of ways   Fresh cow's milk has an average salt concentration of about 0 3 per cent and this provides fluid as well as salt   The workers of South Metropolitan Gas Co , Great Britain (25), are given a saline drink of 0 012 per cent KCl and 0 018 per cent NaCl   The number of heat cramp cases has been reduced since this regulation became effective   Salted drinking water (sodium chloride 0 25 to 1 0 per cent) has been used successfully in prevention of heat cramps among soldiers in the U S Army (26)   Barley water and salted beer are used by certain local groups of colliers in England   It is possible that no salt need be taken between meals if the food is liberally salted at meal times   The necessary amount of salt to prevent cramps is a function of the individual's susceptibility and of the amount lost in the sweat at a given temperature

SUMMARY

This paper is a clinical description of five cases of heat cramps with a chemical study of the blood and urine   The hypothesis is advanced that the etiologic factor is a loss of base, chlorides and water from the body principally by way of the sweat glands without adequate replacement of the same   It is obvious that heat cramps is essentially an occupational disease and an industrial hazard is created by the association of hard work, high external temperatures and profuse sweating

PROTOCOLS

Case 1

Mr J F , a married white man of 42, entered the hospital on June 24, 1932, complaining of vomiting for 3 days and generalized muscle cramps for 12 hours

Past history Born in Nova Scotia, he has lived for the past 28 years in southwestern United States He has never been in the tropics, has never noted any ill effects of heat, and prefers summer to winter He perspires freely in a warm or hot environment and his skin tans easily He came to Boulder City June 6, 1931, and has worked since that time in the Machine Shop situated on the edge of the city He drank at least two quarts of milk daily during the first four months of his employment in the summer of 1931 In the fall his family moved to Las Vegas and he had virtually no milk after that time He is a light meat eater, preferring fruits and vegetables and not especially addicted to the use of salt.

Present illness Three days ago he began to vomit and, while the vomiting has diminished in severity, only a small amount of food and fluid has been tolerated during this period Twelve hours ago cramps in the muscles of his legs first appeared and since then they have caused pain in his arms, hands, and abdominal wall Change of position will generally bring on cramps in the parts moved, although deep pressure or palpation is not painful He voided small amounts of urine twice during the twenty four hours before admission He has continued to perspire freely in spite of the low fluid intake

Physical examination Temperature, 99 6° F pulse, 88, respiration, 16 blood pressure, 130/84 The examination was essentially negative The eye grounds were normal Chvostek's and Trousseau s signs were negative No fibrillary twitchings were seen

Diagnosis Heat cramps

Treatment He was given, during the first 24 hours, two hypodermoclyses of 1500 cc of normal saline and was allowed to drink 3000 cc of milk He received no other food or fluids

Course The cramps ceased after 6 hours He was sent home on the 3d day on a diet containing a minimum of one quart of milk daily He resumed work the third day after discharge Frequent follow ups revealed no muscle soreness or cramps during the next two periods of hot weather

Case 2

Mr W E T , a single white male of 39, entered the hospital June 25, 1932 complaining of right sided headache and weakness in the legs of a few minutes duration

Past history He had served in the Navy during the World War and during the past year had several radical operations on his sinuses in the Veterans Hospital in Los Angeles He had been discharged from the hospital only one month when he was given a job on Hoover Dam He had never lived or worked in a hot climate previously and had worked only two hours the first day of employment when he stopped because of the above complaints His daily intake of milk was over one quart

Present illness He had been working only two hours when he was forced to stop because of a severe right sided headache and a weakness of his legs He did not have any muscle cramps

Physical examination Temperature, 98 4° F pulse, 88 The scars from the previous sinus operations were the only findings of note The reflexes were not altered

Diagnosis   Mild heat prostration
Treatment   He was given, during the 24 hours in the hospital, one hypodermoclysis of 1000 cc and was allowed to drink milk ad libitum
Course   He had no complaints after going to bed   His course through the summer was not followed as he did not return to work

Case 3

Mr H P , a single white man of 42, entered the hospital June 27, 1932, complaining of vomiting and weakness for 12 hours
Past history   He had lived from 1908 to 1910 in the Philippine Islands as a soldier in the United States Army   He had muscle cramps many times when working in the heat   The cramps were transitory and persisted no more than a few minutes with no therapy other than that of stopping work   He did not have any symptoms of heat stroke or heat exhaustion   From 1922 to 1928 he worked in a steel rolling mill in the United States and would have mild cramps at frequent intervals   He came to Boulder City in March, 1932, and has had mild muscle cramps three times since   These were in the calves of the legs or insteps of the feet   He has had no cramps for 3 weeks   His diet includes two glasses of milk daily   A warm climate is preferred to a cold one, his skin burns or tans depending upon the gradation of exposure to the sun and he perspires freely
Present illness   The patient was aware of the relatively high atmospheric temperature on the day preceding admission   He perspired freely and was aware of an increased water intake at work on this day   During the night he vomited several times and felt weak   He did not have muscle cramps
Physical examination   Temperature, 98° F , pulse, 88   Slight tenderness in the epigastrium was the most important physical finding
Diagnosis   Mild heat prostration
Treatment   He was given, during the first 24 hours, two intravenous infusions of 1000 cc of normal saline and was allowed a diet exclusively of milk
Course   He did not vomit after admission to the hospital and was discharged the following day   A follow-up report 6 weeks later showed no symptoms since leaving the hospital

Case 4

Mr W R , a divorced white man of 42, entered the hospital on July 6, 1932, complaining of gas on the stomach and precordial pain radiating to the interscapular region
Past history   Born in Kentucky, he has lived in the west for the past 10 years   For 22 years he has been employed as a miner at frequent intervals In 1919 and 1920 he worked in the Texas oilfields in very hot weather without any ill effects directly attributed to the heat   Fifteen years ago he had a dizzy spell accompanied by slight cardiac pain   This was very mild and he has had no cardiac complaints at any time since   He came to Boulder City in March, 1932 and since that time has worked in the tunnels
Present illness   The past week-end he went on a mild alcoholic bout and returned to work the day of admission   Soon after starting work he had gas on his stomach followed by a short period of delirium   On partially recovering from the delirium he complained of mild precordial pain radiating to the interscapular region and fear of impending death   When he was brought to the hospital he was mildly delirious
Physical examination   Temperature 99 8° F , pulse, 98 respiration, 24

blood pressure 132/84   There was mild dyspnea, precordial hyperesthesia, and the heart sounds were weak but no murmur or rub was heard

Diagnosis   Heat cramps of myocardium?

Treatment   He was given, during the first 24 hours an intravenous infusion of 1000 cc of normal saline in 5 per cent glucose and was allowed to drink 2000 cc of milk   He received no other food or fluids

Course   The patient was discharged after 24 hours and left Boulder City There is no follow up report available

Case 5

Mr R F , a single white man of 33, entered the hospital on July 8, 1932, complaining of severe pain in the calves of his legs for 2 hours

Past history   Born in Tennessee he has lived the past 3½ years in the southwest   He has never complained of any ill effects of heat and prefers summer to winter   He drinks a great deal of water and perspires freely in the heat Since coming to Boulder City 4 months ago he has worked in the tunnels He drank 2 to 3 pints of milk daily until 2 weeks ago, at which time he stopped because of dislike for it   In recent years he has not added salt to his food at the table

Present illness   Ten days ago he had a mild gastric upset from which he believes he has not fully recovered   He drank a small amount of beer over the holiday week end   The night before admission he had slight tingling in the fingers and cramps in the leg muscles   About one hour after starting work on his second shift since the holidays, he became dizzy and vomited   The day was very warm and the profuse sweating was followed by consumption of large amounts of water   The patient complained of such severe pains in the legs that the ambulance attendant reported the case as traumatic

Physical examination   Temperature 101 0° F   pulse, 88 blood pressure, 128/82   A palpable spleen was the only abnormal finding   The reflexes were normal and no tenderness or fibrillary twitchings of the muscles were elicited

Diagnosis   Mild heat cramps

Treatment   A high milk diet was the only order

Course   There was no recurrence of cramps and he was discharged after 3 days   He worked for 2 weeks without any symptoms, but decided he needed a rest so he went to his home in Tennessee for the remainder of the summer

Case 6

Mr G D , a married white male of 38, entered the hospital on July 9, 1932, complaining of muscle cramps and vomiting for 3 hours

Past history   Born in California he has spent a long part of his life in a warm country   From 1920 to 1925 he was in Panama, while in recent years he has been working in Imperial and Death Valleys California, without any ill effects of the heat   He came to Boulder City only 3 days ago and has worked outside on the Arizona Spillway for 2 days

Present illness   The day before admission he felt badly at work but a headache was the only specific complaint   He was slightly constipated and took a laxative with a good result some hours after   He obtained a good rest on the night before admission but felt badly on going to work   Muscle cramps in the arms legs and abdomen forced him to stop work and after vomiting 3 times he was sent to the hospital   On admission he complained of severe headache in addition to the cramps

Physical examination   Temperature, 99 8° F   pulse, 83 blood pressure 118/68   The physical examination was negative

Diagrosis   Severe heat cramps

Treatment   He was given an intravenous infusion of 1000 cc of normal saline in 5 per cent glucose and was allowed a high milk diet

Course   He had no complaints after 6 hours and next morning felt well enough to go back to work   He was discharged on the third day and returned to work the fourth day after admission   For 10 days he was symptom-free on a high salt and high milk diet   On the tenth day he fell while scaling a cliff, injuring his lumbar spine, and he did not return to work the remainder of the summer

Case 7

Mr J C , a single white male of 38, entered the hospital on July 23, 1932, complaining of cramps for 5 hours

Past history   He was born in California and has lived for many years in a warm climate   From 1912 to 1914 he was stationed as a soldier in the Philippines and the only sickness there was dysentery   In 1929 he had mild muscle cramps while working in a hot climate   There was no recurrence   He came to Boulder City the day before admission and had only worked the first shift In the heat he perspires very freely and has a large water intake

Present illness   Three hours after commencing work he began to have muscle cramps, first in the left hand, spreading to the legs and abdominal muscles   He noted slight nausea but no vomiting or diarrhea   There was a slight frontal headache   His water intake was large in amount

Physical examination   Temperature, 98 6° F , pulse, 84, blood pressure, 122/84   There was no muscle tenderness and no twitchings were seen   The biceps reflex was bilaterally increased   The other reflexes were present and responded normally

Diagnosis   Severe heat cramps

Treatment   He was given, shortly after admission, an intravenous infusion of 1000 cc of normal saline in 5 per cent glucose   His diet during the hospital stay was milk alone

Course   The cramps ceased after 4 hours, and he was discharged the next morning   Two weeks after return to work he continued to be free of cramps

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STUDIES OF GASTRIC PEPSIN   I   METHODS OF MEASUREMENT AND FACTORS WHICH INFLUENCE IT

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The study of gastric pepsin has failed to receive adequate consideration for several reasons, but mainly because of the lack of technic for obtaining reliable measurements   We investigated the method originally proposed by Gates (1) and more fully elaborated by Gilman and Cowgill (2), and found it to be accurate and highly satisfactory   In fact, it is more accurate than is needed in clinical practice   We have used this method, with slight modifications, in the investigations reported in this and the following paper   The details of the technic can be obtained from the original article by Gilman and Cowgill (2)   In this method one measures the rate of digestion of gelatin from an exposed and developed photographic film   As digestion proceeds, the silver granules in the gelatin are liberated, and the resulting decrease in opacity of the film is taken as a measure of the digestion which has occurred

TECHNIC

We use, for the digestion, a cell 11 mm in diameter and 1 2 mm in depth turned out of sheet bakelite on a lathe   Its capacity is approximately 0 5 cc   The solution to be tested, diluted with an equal volume of glycine hydrochloric acid buffer of pH 2, is placed in the cell   A small square of the prepared film is then laid over the top of the cell and this is covered by a glass square   The whole is clamped together by means of a spring clothes pin and placed in a water bath the temperature of which is maintained at 25° C   After fifteen minutes the film is removed, washed, and dried, and the change in opacity is determined with a color imeter   The standard for comparison is a freshly prepared suspension of colloidal silver   The index of peptic digestion is obtained by the use of a nomogram as described by Gilman and Cowgill (2)

The Unit of Measurement

We have not found it necessary to conduct the determinations in triplicate because of the close agreement of duplicate determinations Softening of the film due simply to moistening with the solution causes an error of approximately 70 units which must be subtracted from each determination   Since films prepared at different times may differ slightly in this respect, it is well to determine the correction for each lot of film prepared

The unit of pepsin arbitrarily established by Gilman and Cowgill represents the proteolytic activity of a 1   1,000,000,000 solution of a theoretically pure pepsin   They arrived at this unit by assuming that a 1 per cent solution of Armour's 1   10,000 pepsin powder contains 1,000 units

Because of possible variations in the strength of different lots of the commercial pepsin used it is well always to standardize each new lot against the one previously employed   It has been our experience that a solution of commercial pepsin is not sufficiently stable to be used from day to day   The standard must therefore be prepared each time determinations are to be made   The pepsin in the gastric contents of man and dogs is much more stable, and retains its strength for at least a week if kept at ice box temperature

Before taking up a systematic study of gastric pepsin in various diseases it was necessary first to determine the best conditions for obtaining the juice for analysis   First we studied the residuum aspirated in the morning, before taking food or drink, but we found the concentration of pepsin in such juice so variable that single determinations had little value Actually in specimens from twenty-four normal, fasting persons it varied from 5 to 1,930 units, with an average of 388 units   This extreme variability is doubtless due to the variation in the size of the fractions of swallowed saliva, regurgitated duodenal secretion, and gastric juice which make up the material obtained   In subsequent work we used gastric contents removed one hour after the taking of eight arrowroot cookies and 400 cc of water   We examined, in this way, juice from eighty-five persons whom we believed to be normal inasmuch as they had no complaint referable to the upper part of the digestive tract, or disease which might affect gastric secretion   Considerable variation existed in the content of pepsin, the value ranging from 0 to 580 units   The modal or most typical value was about 100 units   The arithmetic mean was distinctly higher than this, due to a few high readings   For the entire group the mean was 145 units, with a standard deviation of 125 units From Figure 1 it can be seen that the men had slightly higher values than the women   This is in accord with the higher acidity in males which has previously been demonstrated (3)   Although the variability in the read-

ings obtained after an Ewald type of meal is high, it is considerably less than that found with juice of fasting subjects

An effort was made to determine the range of variation in repeated estimations in the case of one individual   Eight determinations made on successive days in a case of pseudo ulcer associated with high pepsin readings ranged from 610 to 1,930 units, giving a mean value of 1,175 and a standard deviation of 480 units   In this case seventeen estimations of pepsin in the fasting juice ranged from 410 to 2,530 units, giving a
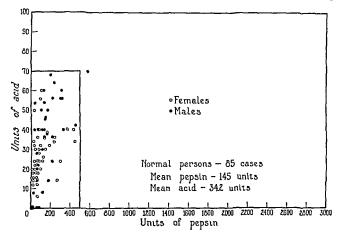
FIG 1   DISTRIBUTION OF CONCENTRATIONS OF ACID AND PEPSIN AFTER EWALD
TYPE OF TEST MEAL IN EIGHTY FIVE NORMAL PERSONS
The brackets indicate normal limits for acid and pepsin

mean of 1,319, and a standard deviation of 592 units   Repeated tests on the gastric residuums from a group of twenty three patients, and a similar study of juice obtained after test meals given to three patients showed somewhat less variability   In the face of such marked variations in the values obtained from one person when examined on several days, it is obvious that before one can call any reading abnormal it must be well beyond the range of normal figures

Effect of Contaminants

Before one can evaluate differences in concentration of pepsin it is necessary to know what effect, if any, is likely to be produced by the presence of a number of common contaminants of the gastric juice   The necessary investigations were made both in vitro and in vivo   The sub

stances which we felt might be of some importance because of their frequent occurrence in gastric contents were bile, bilirubin, mucin, blood and its several constituents such as plasma, serum, erythrocytes and crystalline hemoglobin, protein in the form of egg albumen, and peptones As can be seen in Table 1, all of these substances caused more or less reduction in peptic activity

TABLE 1

The effect of various substances on the activity of pepsin solution *

After adding	Units
Dried bile (0 5 cc)	59
Bilirubin (20 mgm)	92
Mucin (50 mgm)	81
Dried plasma (0 5 cc)	150
Dried plasma (0 5 cc)	127
Dried serum (0 5 cc)	96
Washed erythrocytes, dried (0 1 cc)	100
Crystalline hemoglobin (50 mgm)	152
Egg albumen (20 mgm)	163
Egg albumen (50 mgm)	70
Hard boiled egg in one mass (50 mgm)	272
Hard boiled egg finely divided (50 mgm)	100
Witte's peptone (20 mgm)	132
Witte's peptone (50 mgm)	68

* In each experiment a weighed amount of the substance to be tested was added to 1 cc of a solution of pepsin, the activity of which corresponded to 500 units The figures represent the depressed values obtained after incubation The amount of injury to peptic activity was represented in each case by the difference between the reading obtained and 500

It is often stated in the literature that the presence of blood in the gastric contents will decrease proteolytic activity, and that this is due to an antipepsin Table 2 shows that the ingestion of blood markedly lowered the proteolytic power of gastric contents, but this effect was followed by a stimulation of secretion which soon compensated for the preliminary drops Since a similar effect was produced by raw liver, and also by beef extract, it is doubtful if the decrease of peptic activity in the presence of blood is due to a specific antipepsin The same decrease may be found in vitro after the addition of crystalline hemoglobin or other fairly pure constituents of the blood (Table 1) The degree of reduction in activity seems to be roughly a function of the amount of organic material added The possibility presents itself that the decrease may be due simply to an adsorption which results in the removal of pepsin from solution

As is well known, the effect of raising the pH is to reduce peptic activity, and an alkaline reaction actually destroys much of the ferment The effect of alkali when taken into the stomach of a human being is shown in Table 2, experiment 5 Following the ingestion of two Sippy

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TABLE 2

Experiments in vivo on a patient with a high peptic value

Experiment 1

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	Units
Fasting specimen	1410
Thirty minutes after ingestion of 5 cc of dried cow s blood in capsule	620
Sixty minutes after ingestion of 5 cc of dried cow s blood in capsule	510
Ninety minutes after ingestion of 5 cc of dried cow s blood in capsule	820
One hundred twenty minutes after ingestion of 5 cc of dried cow s blood in capsule	820

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Experiment 2

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Fasting specimen	1850
Thirty minutes after ingestion of 10 cc of human blood	184
Thirty minutes after ingestion of second 10 cc of human blood	910

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Experiment 3

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Fasting specimen	1520
Thirty minutes after ingestion of Liebig extract	484
Sixty minutes after ingestion of Liebig extract	1320
Ninety minutes after ingestion of Liebig extract	810
One hundred twenty minutes after ingestion of Liebig extract	1520

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Experiment 4

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Fasting specimen	950
Thirty minutes after ingestion of 60 grams raw liver	165
Sixty minutes after ingestion of 60 grams raw liver	420
Ninety minutes after ingestion of 60 grams raw liver	514
One hundred twenty minutes after ingestion of 60 grams raw liver	734

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Experiment 5

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Fasting specimen	2040
Thirty minutes after ingestion of two Sippy tablets	180
Sixty minutes after ingestion of two Sippy tablets	1200

tablets, containing a total of 30 grains (2 grams) of calcium carbonate and 20 grains (1 30 gram) of sodium bicarbonate, peptic activity was markedly decreased, and the return to the previous reading was slow   This depressing effect of the alkali was the more striking because the experiment was performed on a subject whose normal peptic activity is unusually marked

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SUMMARY

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It has been found that the Gates method (1) as modified by Gilman and Cowgill (2) gives accurate and reproducible values for the proteolytic activity of a specimen of gastric contents

The variability in readings obtained when successive specimens of fasting residuum from the same subject were used was so great as to destroy their clinical value   More consistent results were obtained with juice obtained after an Ewald type of meal   Standards of normal for pepsin after this type of test meal are published in this paper

Bile, mucin, blood proteins, albumin, peptones, and alkalies markedly reduced peptic activity   The fact that these substances may modify

peptic activity must be kept in mind in making a clinical study of gastric pepsin

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STUDIES OF GASTRIC PEPSIN   II SECRETION OF PEPSIN IN CASES OF DUODENAL ULCER AND PSEUDO–ULCER

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Few articles on concentration of pepsin in the gastric juice of patients with disease of the stomach and duodenum are to be found in recent literature, and the results reported by different observers are not always in accord   We shall consider here only the concentration of pepsin in the gastric juice of persons suffering with the syndrome of peptic ulcer

Wilcox (5), in 1908, reported that the concentration of pepsin in the gastric juice of persons with peptic ulcer and hyperacidity was usually higher, and never lower, than normal   Boas (1), in 1925, and Hirsch-Mamroth and Rindfleisch (3) likewise found that the concentration of pepsin was elevated in the presence of ulcer, provided blood was not present in the sample examined, but they quoted other workers who at times failed to find any increase   Polland and Bloomfield (4) reported that the range of concentration of pepsin was the same in patients with ulcer as in normal persons

We have estimated the concentration of pepsin by a modification of the Gilman Cowgill method (2), described in the preceding article (6)   As an arbitrary standard of measurement a 1 per cent solution of Armour's 1   10,000 commercial pepsin was assumed to contain 1,000 units

In the greater part of the investigation we examined gastric contents removed one hour after the ingestion of a test meal which consisted of eight arrowroot cookies and 400 cc of water   We also determined the content of pepsin in the juice removed from the stomach in the morning, before breakfast   Although the same general conclusions may be drawn from studies of contents removed after a test meal and of juice from the fasting stomach, it is necessary to consider the results obtained with the two technics separately because of differences observed in the absolute values for pepsin

Duodenal ulcer

The diagnosis of duodenal ulcer was made in 274 cases on the basis of the history and fluoroscopic examination   In fifty seven cases the

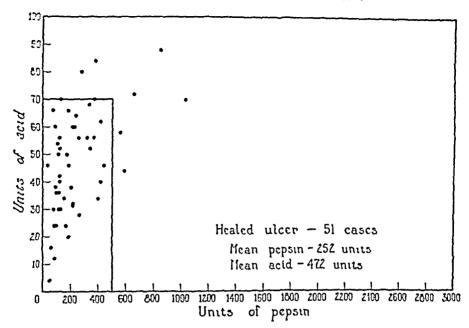[1] Fellow Josiah Macy, Jr , Foundation, on duty at The Mayo Foundation, Rochester Minnesota

FIG 1   THE DISTRIBUTION OF ACID AND PEPSIN IN HEALED DUODENAL ULCER
The bracket indicates limits of normal

diagnosis was verified at operation   In fifty-one of the 274 cases a diagnosis of duodenal ulcer had been made, the duodenal cap was still deformed, but symptoms of ulcer had not been present for a number of years   Most of the pepsin readings in these cases of presumably healed ulcer (Fig 1) fell within normal limits, but the arithmetic mean was 252 units as compared with 145 units for the normal group (Table 1)

TABLE 1
Ewald test meal

	Number of cases	Pepsin in units		Free acid in terms of cc of normal sodium hydroxide	
		Mean	Standard deviation	Mean	Standard deviation
Normal	85	145	125	34 2	12 8
Duodenal ulcer entire group	274	388	343	55 5	18 0
Healed	51	252	209	47 2	18 8
Mild	29	292	334	54 9	18.3
Moderate	92	332	312	55 3	16 2
Severe	28	824	505	62 2	17 2
Surgical	77	423	300	58 9	17 0
Hemorrhagic type	17	443	272	63 8	14 2
Multiple ulcers	15	398	273	61 1	15 8
Penetrating type	23	407	238	62 1	16 4
Obstructing type	11	195	116	51 1	14 2
Gastric ulcer	13	230	173	46.3	19 6
Ulcers recurring after operation	18	476	341	55 2	20 8
Pseudo-ulcer	47	467	384	49 6	18 0

In the remaining 223 cases the patients complained of active symptoms of duodenal ulcer   The mean value for pepsin presented by these patients was 419 units, which is approximately two and five-tenths times the mean value for normal persons   The readings for pepsin in many cases fell far outside the normal limits

We next looked to see if there might be a difference between the average concentration of pepsin in cases of mild and severe ulcer   The difficulty, of course, was to find satisfactory criteria for division of the cases into groups   We finally decided to classify the cases according to the treatment which was required   We classified as mild those cases in which the symptoms were so trivial and so easily controlled that no treatment of any kind was indicated, and as moderately severe that large group in which medical treatment gave relief   The mean value for pepsin in the group with mild symptoms was 292 units, only slightly higher than that found among patients with healed ulcer, in the group of patients with more serious trouble it was 332 units (Fig 2)
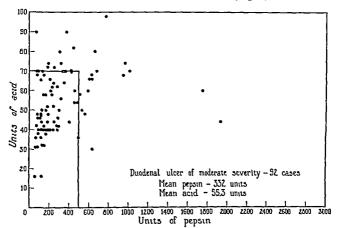
FIG 2   THE DISTRIBUTION OF ACID AND PEPSIN IN DUODENAL ULCER OF
MODERATE SEVERITY
The bracket indicates limits of normal

We considered as severe those cases in which symptoms persisted in spite of the most careful treatment in the hospital   Almost all of the values for pepsin in this group fell outside the limits of normal (Fig 3), and in many instances very high readings were obtained   The mean value was 824 units, more than two and five tenths times that seen in the ordinary case of duodenal ulcer, and nearly six times that seen in normal

persons   In this group we did not include cases in which there was hem-
orrhage or a tendency to perforation because such complications usually
constituted indications for surgical treatment   We did include many of
those cases in which the patient was of a tense, nervous, "go-getter"
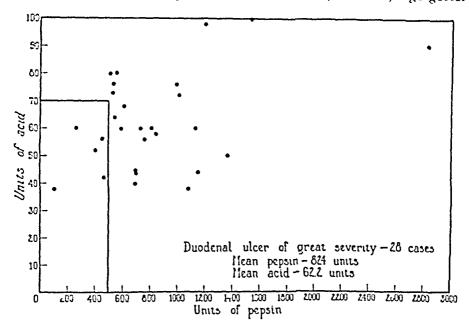
FIG 3   THE DISTRIBUTION OF ACID AND PEPSIN IN SEVERE DUODENAL ULCER
The bracket indicates limits of normal

type, the sort of patient whom gastro-enterologists and gastric surgeons
have come to recognize as a poor subject for either medical or surgical
treatment   It was in this type of case that the highest readings were
obtained
    Seventy-seven patients were advised to submit to surgical treatment
either because of the presence of some complication or because of the
chronicity or intractability of the disease   In this group the average
value for pepsin was 423 units, or about 100 units higher than the average
for the medically treated patients
    On analyzing the surgical cases we found a hemorrhagic type of ulcer
in seventeen, multiple ulcers in fifteen, and penetrating ulcers in twenty-
three cases   In these three groups the values for pepsin were somewhat
higher than among patients who had a less serious form of the disease
The mean for bleeding ulcers was 443 units, for multiple ulcers it was 398
units, and for penetrating ulcers it was 407 units   Among the group of
patients treated surgically there were eleven with some cicatricial obstruc-
tion at the pylorus   In most of these cases the ulcer was chronic, in
some it appeared to be healed   In the cases in which there was moderate

obstruction the mean value for pepsin was even lower than in the group of patients classed as having healed ulcers, namely, 198 units. It should be noted here that all cases of ulcer in which there was gross retention of gastric contents were excluded from this study. This was to avoid the complication of marked dilution of the gastric juice.

Fasting juice. We estimated the concentration of pepsin in the fasting juice of forty patients with duodenal ulcer. They were divided into two groups first, those with mild, and second, those with intractable, ulcer. It was found that the twenty four who responded readily to treatment had an average value for pepsin of 537 units, as compared with 388 units for normal persons, those with severe symptoms had an average of 1,592 units (Table 2). It is evident that the pepsin in the fasting juice

TABLE 2

Fasting juice

Diagnosis	Male	Female	Pepsin units		Free HCl*	
			Mean	Standard deviation	Mean	Standard deviation
Normal	13	11	388	524	13	19
Moderately severe ulcer	18	6	537	617	27	24
Severe ulcer	16		1592	1020	43	23

* In terms of cc of normal sodium hydroxide per liter

with the different types of ulcer varies in the same way that it does in the juice after a test meal, except that the actual values are higher and the individual variation is greater

Pseudo ulcer

A study was made of values for pepsin in a group of forty seven patients who had symptoms sufficiently suggestive of ulcer to justify gastric analysis and fluoroscopic examination of the stomach. In every case the presence of atypical features in the history and the inability of the roentgenologist to detect any deformity in the stomach or cap caused the clinicians to make a diagnosis of pseudo ulcer. The spot diagram (Fig 4) shows that the distribution of values for pepsin is similar to that in cases of ulcer and that some figures are so high as to resemble closely those found in the presence of the most intractable ulcer.

In many of the cases there was close correlation between the concentration of pepsin in the stomach and the severity of the symptoms. Just as in the case of patients with actual ulcer, there was striking correlation between the amount of pepsin and the degree of tenseness and nervous activity shown by the individual.

In six cases in which the diagnosis of pseudo-ulcer was confirmed by the inability of the surgeon at the time of operation to find an ulcer, determinations of pepsin made on fasting juice disclosed an average value of 880 units as compared to the normal mean fasting value of 388 units
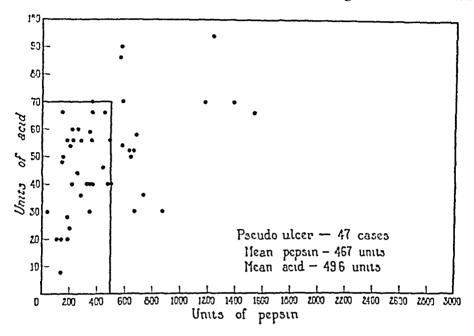
Fig 4   Distribution of Acid and Pepsin in Pseudo-Ulcer
The bracket indicates limits of normal

Gastric ulcer

The diagnosis of gastric ulcer was made in thirteen cases on the basis of roentgenologic examination or surgical exploration   The mean value for pepsin was 230 units, which is slightly higher than normal

Recurrent and gastrojejunal ulcers

There were eighteen patients who, after an operation for relief of peptic ulcer, returned with a new ulcer, either in the duodenum or at the stoma made by gastro-enterostomy   The mean value for pepsin was 476 units   This figure is about one-third higher than that found by us in examination of patients with duodenal ulcer for whom operation was advised   The fact that in many of these cases the gastric juice was diluted by intestinal juice regurgitating through the gastro-enteric stoma makes it seem probable that the secretion of pepsin was even greater than the figures would indicate   It is of interest that the remaining part of the stomach of one of these patients who had undergone extensive gastric resection secreted enough pepsin to push the concentration up to 850 units

The study of pepsin is of more clinical value than the study of acid

We were led to a study of gastric pepsin by the fact that measurement of acid has so far been of so little decisive clinical value   The question now is   Will the differences which we have found in the secretion of pepsin in different diseases be of any more value than are the measurements of acidity?   Several of the early workers felt that there is such a high degree of correlation between the acid and the ferment that little advantage can be gained from determining pepsin except in those cases in which it is desirable to distinguish between achlorhydria and true achylia   Our observations lead us to disagree with this view   Although it is true that high concentrations of pepsin are commonly associated with hyperacidity, and low concentrations with hypoacidity, the rule is by no means invariable, as the coefficients in Table 3 show

TABLE 3

Relation between concentration of pepsin and free hydrochloric acid in gastric juice

	Correlation coefficient
Fasting juice	
Normal persons	0.72 ± 0.11
Patients with duodenal ulcer	0.85 ± 0.12
Juice obtained after Ewald test meal	
Normal persons	0.51 ± 0.08
Patients with duodenal ulcer	0.65 ± 0.13
Juice obtained after stimulation by histamine	
Patients with duodenal ulcer	0.60 ± 0.04

The fact that these coefficients are far from unity shows that often the acid titer and the amount of pepsin do not vary in the same way or to the same degree   When this happens the way is left open for the possibility that a study of the pepsin, another constituent of the gastric juice, will give information of clinical value which was not supplied by titration of the acid alone

A glance at Figure 5 reveals that although there are definite differences between the mean acidity of normal persons and that of patients with mild and with severe ulcer the distributions overlap so closely that in only a few cases will the physician be able to prophesy, from the gastric acidity alone, which ulcers will be hard to treat   On turning now to the curves representing values of pepsin in the three groups of cases, one sees that there is less overlapping of the distributions and when one obtains a reading higher than 800 or 900 units in a case of ulcer, one can be almost certain that the ulcer will be difficult to cure   Unfortunately it is not possible with the help of determinations of pepsin to distinguish patients with pseudo ulcer from those with real ulcer

It should be noted, also, that whereas it is almost impossible for anyone to have a free acid titer more than twice the normal average,
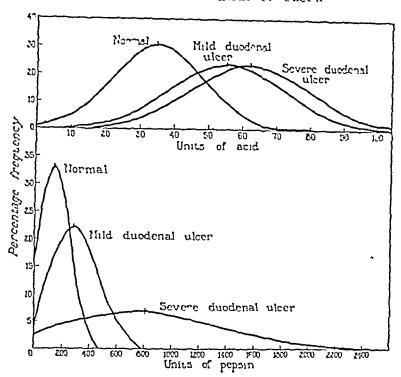
FIG 5   DISTRIBUTION OF PEPSIN AND ACID IN NORMAL PERSONS AND IN PATIENTS WITH MILD AND SEVERE ULCER

readings for pepsin occur from fifteen to twenty times the normal average It is this widening of the distribution of the measurements made in cases of disease, a widening that carries so many of the figures beyond the possible range of normal, which makes us hopeful that the test will have decided value in clinical practice

SUMMARY

Values for pepsin are usually increased in the presence of peptic ulcer, and there is a high degree of correlation between the amount of the ferment and the severity and intractability of the symptoms Unfortunately the determination cannot be used to prove the presence of ulcer because very high values for pepsin are commonly found in cases in which patients are tense and nervous, and present a syndrome resembling that of ulcer Its main value, therefore, seems to be in estimating the prognosis in cases of known ulcer

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STUDIES OF UREA EXCRETION VIII THE EFFECTS ON THE UREA CLEARANCE OF CHANGES IN PROTEIN AND SALT CONTENTS OF THE DIET

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In the study of the effects of protein rich and protein poor diets on nephritic kidneys, relatively little attention has so far been paid to the changes in functional activity   Indications suggesting that such changes exist are to be found in the observations of Addis and Drury (1) that the "maximum urea clearance" can be slightly but definitely increased by a meal of protein-rich food, and in the recently published findings of Jolliffe and Smith (2) that in dogs fed on a cracker meal diet low in protein, the urea clearance is markedly lower than on a high protein diet

It appeared desirable, therefore, to obtain more definite information concerning the effects of protein on the function of human nephritic kidneys   As criterion of renal function, the standard urea clearance of Möller, McIntosh and Van Slyke (5) has been employed   The cases presented are unselected, except that those were discarded which showed evidence during the preliminary observational period that activity of the nephritic process was causing a progressive lowering of renal function at the time, and also a few in which infections or exacerbations occurred during the experimental period and were likely to change the renal function   These two types of cases were excluded because they were subject to such rapid changes that it would have been difficult to tell whether functional changes under different regimes were due to change in regime or in renal pathology   The patients used were those in which the renal condition appeared to be in a fairly steady state

In order to obtain evidence of the effect of salt also on the urea clearance, similar studies were carried out with diets containing large and minimal amounts of sodium chloride   The salt variation had no significant influence on the urea clearance   We shall therefore not detail this part of the work, but merely record its negative results   In one instance the ingestion of 65 grams of sodium chloride and its subsequent excretion at high concentration in the urine had no detectible influence on the urea clearance   It was not found possible, by feeding diets poor in salt, to reduce the plasma chloride to such low concentrations as are
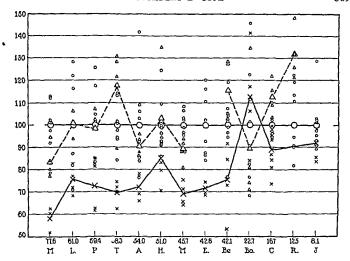

FIG 1 EACH COLUMN OF SYMBOLS SHOWS STANDARD UREA CLEARANCE VALUES FOR AN INDIVIDUAL WHOSE MEAN STANDARD UREA CLEARANCE ON NORMAL DIET (ABOUT 75 GRAMS PROTEIN PER DAY) IS RECORDED ALONG THE LOWER MARGIN

Ordinates represent clearance values expressed for each subject as percentages of his average clearance on a diet with 75 grams of protein

o = values obtained on normal diet (75 grams protein per day),

⊙ = mean of these values,

× = values obtained on low protein diet (40 grams protein per day),

× = mean of these values,

△ = values obtained on high protein diet (120 grams protein per day),

△ = mean of these values

per minute, as shown by the small crosses When the daily protein intake was increased to 120 grams the standard clearance rose once more to be tween 77 per cent and 94 per cent of the original mean, i e to between 59 7 and 73 0 cc per minute, the individual clearances being indicated by the small triangles

The figure shows that when renal function is unimpaired, or only slightly impaired by the nephritic lesion, as in the first 9 cases, the urea clearance is reduced by from 15 to 40 per cent by diminishing the protein intake from 75 grams to 40 grams per day Of the remaining cases, with seriously impaired renal functions, two showed a slight reduction of the urea clearance, about 10 per cent, and one was exceptional in having a urea clearance higher on the low protein than on a protein rich diet No

said by many French writers to cause a nitrogen retention   Diets which reduced the urinary NaCl output to less than 1 gram per day, however, were without effect on the urea clearance

METHODS

The usual methods of estimating and calculating the clearance have been employed (5)   Urea in both blood and urine has been estimated gasometrically by the method of Van Slyke (7), in the former in 1 cc samples of whole blood obtained by venipuncture, in the latter in a volume of urine appropriate to the urea concentration anticipated   Duplicate analyses made in all cases agreed within 1 per cent

After admission to hospital and routine investigation each patient was kept for a week or more on a normal diet containing about 75 grams protein per day   During this time urea clearance determinations, usually eight in number, were made with the patient in bed, for the dual purpose of obtaining average values for the individual on a normal diet, and of making sure that no marked spontaneous change in the urea clearance was taking place   A diet containing 40 grams of protein per day was then given for at least five days before fresh clearance determinations were made   These having been obtained, the protein of the diet was once more increased either to the original 75, or in most cases to 120, grams per day   Rise of the urea clearance during this third period in each of the recorded cases (except the anomalous Bo) was taken as evidence that the depressed clearance in the second period was indeed associated with the low protein diet, and was not an expression of a progressively destructive nephritic process active at the time

RESULTS

Thirteen cases of nephritis have been successfully examined in this manner, and the results are indicated graphically in Figure 1   All the clearance values obtained on a given individual are plotted in a single column   The thirteen columns thus represent the thirteen cases studied and are arranged in order of decreasing renal function   The figure at the bottom of each column is the mean standard urea clearance for that individual on a normal diet, expressed in cubic centimeters of blood per minute   The individual clearance values obtained have been plotted as percentages of this figure   Thus in each case an ordinate value of 100 per cent represents the average standard urea clearance for that subject on a normal diet   To take an example, the first column represents observations on the patient M   On a normal diet his mean standard urea clearance was 77 6 cc per minute   Individual clearance determinations, plotted as small circles, varied from 112 per cent to 79 per cent *of this value*   On a low protein diet the urea clearance fell to between 51 per cent and 62 5 per cent of the original 77 6 cc , i e to between 39 6 and 48 5 cc

rise which followed urea ingestion, and was falling, and that the amount of urea formed from the food or glutamic acid was insufficient to check the fall in blood urea   It appears probable, therefore, that the stimulus to increased blood urea clearance was something other than urea itself

In this connection it is of interest that the hypertrophy of the kidneys, which many workers have produced by feeding a high protein diet, could not be fully duplicated by Osborne, Mendel, Park and Winternitz (6) nor by MacKay, MacKay and Addis (4), by feeding urea alone, a fact which tends to suggest that such hypertrophy also is related to the stimulating effect of products other than urea formed from a protein diet

There is, it is true, one gap in the experimental evidence required to confirm the application of this explanation to our results   All the data available on men have been obtained by starting with subjects in ordinary protein nutrition, with the usual blood urea nitrogen levels of 10 to 16 milligrams per 100 cc   To obtain by urea administration the same blood urea changes that are produced by increasing the protein intake from 40 grams to 75 or more grams, one would need to use subjects who had previously been on a diet containing as little as 40 grams of protein   If urea administration to such subjects did not stimulate a rise in the clearance, it would appear clear that the increased clearances obtained in our subjects by increased protein administration were not due to the accompanying rise in blood urea

<div align="center">SUMMARY</div>

The urea clearance was not affected, in either normal or nephritic subjects, by variations in the sodium chloride content of the diet

Raising the protein content of the diet from 75 grams to 120 grams was also without consistent effect on the urea clearance

However, lowering the protein intake from 75 grams to 40 grams was accompanied by depression of the clearance in 11 out of 12 subjects *with normal or nearly normal renal function*   In these subjects the clearance on the low protein diet was only 60 to 80 per cent as great as on the 75 gram diet

In three patients with renal function *below half normal*, the clearance on low protein remained 90 per cent or more of the clearance on the 75 gram diet

In interpreting subnormal urea clearances as measures of renal function, allowance should be made for the fact that on very low protein diets the lower limit of normal variation may extend down to 50 per cent of the usual normal average, instead of to the 70 per cent minimum found under ordinary conditions (5)   In subjects with markedly depressed function, however, the clearance appears to be relatively fixed, and little influenced by protein restriction

explanation of this anomalous case is offered   In the remaining case, that of R, the low protein diet was not administered, as her plasma proteins were low and it was felt that such a regime would provoke a recurrence of the edema to which she was liable   This case is included, however, because she showed a well marked rise in her urea clearance when the protein intake was increased to 120 grams per day

The effect of high protein regime was more irregular than that of the low   Of the eleven cases which had the high protein intake during the third period, the clearances of four rose to well above the figures for the first or moderate (75 grams) protein diet period, those of three others returned to the original values, and those of a further three rose well above the low protein diet figures, but failed to return completely to the original level   The remaining case Bo was again anomalous showing an effect precisely the reverse of the other twelve, her clearance being reduced on the high protein diet

DISCUSSION

In 11 out of the 12 nephritic patients who have been examined a definite reduction in the urea excretory activity of the kidneys has been demonstrable on a low protein diet, when this activity has been estimated by the standard urea clearance test

In this respect our data confirm the observations of Keutmann and McCann (3) which appeared when our experimental work was nearing completion   Keutmann and McCann studied 4 cases of hemorrhagic Bright's disease, and found that the urea clearance was usually higher on high protein than on low protein diets

In our cases, the effect of low protein diet in reducing the urea clearance is relatively less when the renal activity is already seriously impaired by disease   This fact is in accord with other evidences that the kidney shows a tendency to diminishing variability of activity as the nephritic lesion progresses   Possibly in this instance it is due to retained metabolites stimulating the kidney to a sustained maximal effort

In all cases the rise in urea clearance caused by increasing the protein intake was accompanied by a rise in the blood urea concentration   However, it appears probable that the kidneys are stimulated by some protein metabolite other than urea   Moller, McIntosh, and Van Slyke (5) found that, when the urea nitrogen content of the blood was increased from the usual normal range of 10 to 16 mgm per 100 cc to two or three times as much by feeding urea, the clearance value was not affected   Addis and Drury (1) found that, when the blood urea was first similarly raised by urea ingestion, and later a protein meal or 20 grams of glutamic acid was taken, the meal or amino acid caused a definite decrease in the clearance   The increase in clearance after food or glutamic acid occurred despite the facts that the blood urea had some hours before passed the peak of the

rise which followed urea ingestion, and was falling, and that the amount of urea formed from the food or glutamic acid was insufficient to check the fall in blood urea   It appears probable, therefore, that the stimulus to increased blood urea clearance was something other than urea itself

In this connection it is of interest that the hypertrophy of the kidneys, which many workers have produced by feeding a high protein diet, could not be fully duplicated by Osborne, Mendel, Park and Winternitz (6) nor by MacKay, MacKay and Addis (4), by feeding urea alone, a fact which tends to suggest that such hypertrophy also is related to the stimulating effect of products other than urea formed from a protein diet

There is, it is true, one gap in the experimental evidence required to confirm the application of this explanation to our results   All the data available on men have been obtained by starting with subjects in ordinary protein nutrition, with the usual blood urea nitrogen levels of 10 to 16 milligrams per 100 cc   To obtain by urea administration the same blood urea changes that are produced by increasing the protein intake from 40 grams to 75 or more grams, one would need to use subjects who had previously been on a diet containing as little as 40 grams of protein   If urea administration to such subjects did not stimulate a rise in the clearance, it would appear clear that the increased clearances obtained in our subjects by increased protein administration were not due to the accompanying rise in blood urea

SUMMARY

The urea clearance was not affected, in either normal or nephritic subjects, by variations in the sodium chloride content of the diet

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In three patients with renal function *below half normal*, the clearance on low protein remained 90 per cent or more of the clearance on the 75 gram diet

In interpreting subnormal urea clearances as measures of renal function, allowance should be made for the fact that on very low protein diets the lower limit of normal variation may extend down to 50 per cent of the usual normal average, instead of to the 70 per cent minimum found under ordinary conditions (5)   In subjects with markedly depressed function, however, the clearance appears to be relatively fixed, and little influenced by protein restriction

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THERMAL CHANGES IN PERIPHERAL VASCULAR DISEASE DURING SYMPATHETIC GANGLIONECTOMY UNDER GENERAL ANESTHESIA

By WINCHELL McK CRAIG, BAYARD T HORTON
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A great deal of confusion has existed regarding the differential diagno sis of Raynaud s disease, thrombo angitis obliterans, and arteriosclerosis Raynaud's disease affects, predominantly, young women, and because it is caused by vasospasm is completely relieved in the uncomplicated case, by sympathetic ganglionectomy   It need not be confused with thrombo angitis obliterans and arteriosclerosis with occlusion   Arteriosclerosis with occlusion is usually found among elderly people, and since vaso spasm is rarely, if ever, an element in the condition, it is not affected by sympathetic ganglionectomy   *Thrombo angitis obliterans* practically always affects young men, has an element of vasospasm, and many of the patients can be satisfactorily treated by sympathetic ganglionectomy Simple tests have been sought which would aid in differential diagnosis and which, by revealing the degree of vasodilation possible, would help to determine the suitability of these cases for operation, and, in some de gree, indicate the probable prognosis   Observations made on patients undergoing sympathetic ganglionectomy under general anesthesia re vealed certain thermal changes which may prove of value in this connec tion

With the use of general anesthesia, administered by inhalation, it was noted that extremities which were not denervated were temporarily re lieved of vasomotor spasm, and that there was definite postoperative clinical improvement of these extremities   It was difficult to interpret this temporary improvement, but it was thought for a time that the entire sympathetic nervous system was reflexly or indirectly affected by opera tion on, or manipulation of, any part   Adson (1) ventured the sugges tion that the general anesthetic probably influenced the entire sym pathetic nervous system by affecting the vasoconstrictor center in the diencephalon   Morton and Scott (6) measured the changes in cutaneous

temperature caused by different anesthetic agents and called attention to the different temperature gradients in the extremities of normal people They suggested the use of general anesthesia as a means of selecting cases suitable for sympathetic ganglionectomy, but concluded that it was not satisfactory   Sheard, Rynearson and Craig (9) have shown experimentally that in dogs under anesthesia by inhalation, generalized vasodilation occurred and that this was not influenced by operation on the sympathetic system   Also they showed that under general anesthesia the changes in the temperatures of intact and of sympathectomized extremities, with variations in environmental temperature, were similar

Herrick, Essex and Baldes (5), using a modification of the thermo-stromuhr method of Rein, have shown that following unilateral sympathetic ganglionectomy on dogs, the flow in the femoral arteries on the sympathectomized side was about twice as great as that on the intact side There was no outstanding difference between the readings of the two sides when, following the operations, these animals were placed under ether anesthesia   Hence, it may be concluded that during surgical anesthesia by ether, there develops vasodilation of the vessels of the extremities as great as that following sympathetic ganglionectomy

Thus, it would seem that during operations on the sympathetic nervous system under anesthesia by inhalation, there occurred certain physiologic changes which could be traced directly to the anesthetic, and which were not altered by removal of ganglia   By observing the changes in cutaneous temperature during operations under general anesthesia, certain important facts were obtained which we believe will prove of diagnostic and prognostic value in treating cases of peripheral vascular disease

This study consisted of continuous observations of surface temperature of the extremities of subjects with Raynaud's disease and thrombo-angitis obliterans during the course of thoracic and lumbar sympathetic ganglionectomy   Repeated readings of temperature were made preoperatively with the subjects at rest and with the room temperature at approximately 24° C   A portable electromotive thermometer, which is a modification of the ensemble described by Sheard (8), was used throughout the investigations   The instrument used carried four thermocouples for application to the hands and feet   By means of a rotary switch, any thermocouple could be placed in the circuit and the temperature indicated by each of the thermocouples could be read quickly and accurately on the micro-ammeter calibrated in degrees Centigrade   The thermocouples were left in place during the entire course of the operation, so that graphic records were obtained of the changes in surface temperature of the extremities as influenced by anesthesia alone and by the interruption of the sympathetic innervation to the extremities   The operations were carried out under general anesthesia with nitrous oxide, oxygen and ether, or ethylene, oxygen and ether   Regardless of induction and maintenance

with gas, oxygen, or ether administered by the drop method, the relative changes in the temperature curve were the same   Observations of the blood pressure and pulse were made during this period   For a control study, similar observations of changes of surface temperature were made on subjects during the course of operation for other conditions

The rise in surface temperature of the extremities of an apparently normal subject is illustrated in Figure 1 and represents a normal response
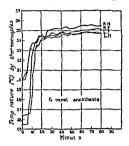

FIG 1   COURSE OF THERMAL CHANGES IN HANDS AND FEET OF A PATIENT WITH APPARENTLY NORMAL VASOMOTOR REACTION TO GENERAL ANESTHESIA
R H , right hand   R F , right foot   L F , left foot, and L H , left hand

of vasodilation   There was a relatively sharp and uniform rise in the surface temperature of all four extremities, within the first ten minutes after the induction of anesthesia   The maximal rise of surface temperature occurred within twenty to thirty minutes and remained constant for one hour, the pulse rate varied from 70 to 80 beats each minute, and the systolic blood pressure varied from 122 to 130 mm Hg and the diastolic, from 60 to 74 mm Hg

The marked similarity between the rise in surface temperature of the extremities in a subject with Raynaud's disease and that of the normal person is illustrated by Figure 2a   The same sharp rise in surface temperature occurred in all four extremities immediately after induction of anesthesia, and reached its maximum within twenty to twenty five minutes   Practically no change was observed in the surface temperature of the extremities after this period   This is what one would expect in a case of Raynaud's disease, which is a primary vasospastic disturbance involving the vessels of the extremities   It is interesting to note that no change in the surface temperature of the lower extremities was observed following interruption of the sympathetic nerve supply   In other words, the maximal rise in surface temperature had occurred immediately following induction of the anesthesia, and severance of the sympathetic nerve supply caused no additional rise in surface temperature   This is of particular interest because it was formerly a clinical impression among certain

workers that immediately following interruption of the sympathetic nerve supply to a given extremity, there was always a sharp rise of surface temperature and an increase in flow of blood in that extremity

The rise in surface temperature of the extremities of subjects with thrombo-angitis obliterans varied considerably among different persons
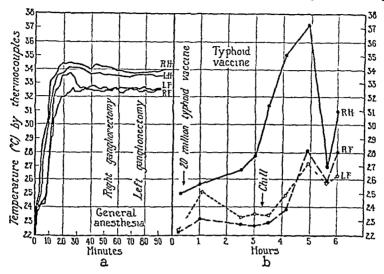

FIG 2   INCREASE IN SURFACE TEMPERATURE OF THE HANDS AND FEET OF A WOMAN AGED TWENTY-THREE YEARS WHO HAD RAYNAUD'S DISEASE

Pulsations in the peripheral vessels were normal   a   The rise with general anesthesia   b   The rise obtained with intravenous injection of typhoid vaccine   Rise in mouth temperature was 1 1° C   R H , right hand, R F , right foot, L F , left foot, and L H , left hand

and corresponded clinically to the degree of occlusive vascular disease present (Figs 3a, 4a, 5a and 6a)   In thrombo-angitis obliterans, one is dealing primarily with an occlusive vascular disease which involves the extremities   Vasospastic disturbance in the collateral circulation is invariably present to some degree   Interruption of the sympathetic nerve supply to the affected extremities permits the maximal flow of blood through the collateral circulation, but it is obvious that the occluded vessels are not affected by this surgical procedure   In cases in which there is a small degree of occlusion and a large degree of superimposed vasospasm in the collateral circulation, the rise in surface temperature of the extremities parallels that observed in cases of Raynaud's disease   In moderately advanced cases of thrombo-angitis obliterans, uniform rises in surface temperature of all four extremities were not observed, but in early cases the rise in surface temperature of the extremities may closely simulate that observed in Raynaud's disease, as illustrated in Figure 3a

It is interesting to compare the various rises in temperature obtained with intravenous administration of typhoid vaccine in a given case and
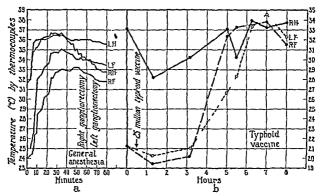
FIG 3   VARIATION IN SURFACE TEMPERATURES OF THE HANDS AND FEET OF
A MAN AGED THIRTY TWO YEARS WHO HAD THROMBO ANGIITIS OBLITERANS

Pulsations in the right and left ulnar, radial femoral and popliteal arteries
were normal   In other arteries pulsations were reduced as follows in the
right dorsalis pedis, 80 per cent in the left dorsalis pedis, 65 per cent, in the
right posterior tibial, 35 per cent, and in the left posterior tibial 25 per cent
a The rise with general anesthesia   b The rise obtained with intravenous
injection of typhoid vaccine   Rise in mouth temperature was 0 5° C   R H ,
right hand R F , right foot L F , left foot, and L H , left hand

FIG 4   INCREASE IN SURFACE TEMPERATURE OF THE HANDS AND FEET OF A
MAN AGED FORTY YEARS WHO HAD THROMBO ANGIITIS OBLITERANS

The right and left ulnar radial and femoral arteries pulsated normally
Pulsations in both popliteal arteries were reduced approximately 50 per cent
in the right and left dorsalis pedis and posterior tibial arteries pulsations were
absent   Circulatory insufficiency was more marked in the left than in the
right foot   a The rise with general anesthesia   b The rise obtained with
intravenous injection of typhoid vaccine   Rise in mouth temperature was
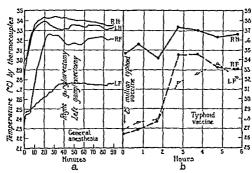1 3° C   R H , right hand R F , right foot L F , left foot, and L H , left hand

that obtained following induction of general anesthesia   We have pre-
viously been of the opinion that similar temperature curves could be ob-
tained   This, however, proved not to be true, for there was marked dis-
similarity between the curves obtained following intravenous administra-
tion of typhoid vaccine and that following induction of general anes-
thesia (Figs 2b, 3b, 4b, 5b and 6b)   This in part may be due to the fact
that some subjects are either refractory to or react poorly to intravenous
administration of typhoid vaccine   The maximal flow of blood to the
extremities occurred following both procedures, but it occurred more
quickly after general anesthesia than after intravenous administration of
typhoid vaccine   With general anesthesia, fewer fluctuations were ob-
served after the surface temperature had reached its maximum   It
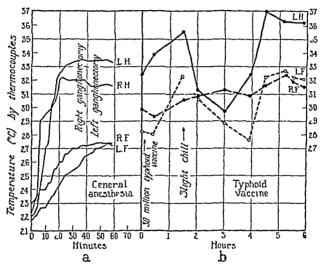

FIG 5   VARIATION IN SURFACE TEMPERATURE OF THE HANDS AND FEET OF A
MAN AGED TWENTY-NINE YEARS WHO HAD THROMBO-ANGIITIS OBLITERANS
     Pulsations in the right and left ulnar, radial, and femoral arteries, and in
the right popliteal arteries, were normal, those in the left popliteal artery were
reduced 25 per cent   Pulsations in the right and left dorsalis pedis arteries were
reduced 40 per cent, and the right and left posterior tibial arteries were occluded
The circulatory insufficiency was the same in both feet   a The rise with
general anesthesia   b The rise obtained with intravenous injection of typhoid
vaccine   Rise in mouth temperature was 2 2° C   R H , right hand, R F ,
right foot, L F , left foot, and L H , left hand

was also evident that the maximal flow of blood to the extremities follow-
ing general anesthesia occurred within twenty or thirty minutes and was
not increased after that time   The fluctuation in the curves of surface
temperature following intravenous injection of typhoid vaccine is ac-
counted for, in part at least, by the chill which so frequently develops
Brown originally used typhoid vaccine intravenously to determine what
he designated "the vasomotor index "   He and Adson (2, 3) used this

method for selecting subjects with Raynaud's disease and thrombo-
angiitis obliterans for sympathetic ganglionectomy   It has proved to be
a satisfactory means of selecting cases for this surgical procedure   In
addition, the intravenous administration of typhoid vaccine or "fever
therapy" undoubtedly represents the most important medical method
of treatment for these diseases   This is particularly true with reference
to thrombo angiitis obliterans

Other methods for selecting cases for sympathetic ganglionectomy are
spinal anesthesia, which is applicable only to the lower extremities, and
paravertebral injection of procaine in the cervicothoracic region for the
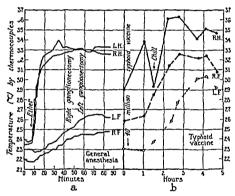upper extremities   Oral administration of alcohol, intramuscular injec-

FIG 6   VARIATION IN SURFACE TEMPERATURE OF HANDS AND FEET OF A MAN
   AGED THIRTY TWO YEARS WHO HAD THROMBO ANGIITIS OBLITERANS
   Pulsations in the right ulnar, radial, and femoral arteries, and in the left
radial and femoral arteries, were normal   Pulsations were absent in the other
palpable vessels   Circulatory insufficiency was more marked in the right than
in the left foot   a The rise with general anesthesia   b The rise obtained
with intravenous injection of typhoid vaccine   Rise in mouth temperature
was 1° C   R H , right hand R F , right foot L F , left foot, and L H , left
hand

tion of acetylcholine, application of heat, and many other procedures also
have been used with varying degrees of success   The procedure of ob
serving surface temperatures of the subject under anesthesia affords a
satisfactory method for investigating the amount and extent of vasospas
tic disturbances particularly in the extremities of subjects with Ray-
naud's disease and thrombo angiitis obliterans   If satisfactory rises in
the surface temperature of the involved extremities are not observed
after induction of anesthesia, it is evident that an additional rise will not
develop after interruption of the sympathetic innervation   The

observations were made in investigating cases of vasospastic scleroderma and chronic infectious arthritis   Thermal changes under general anesthesia give valuable information from the prognostic standpoint in subjects with thrombo-angiitis obliterans   Accurate information can be obtained regarding the extent of the occlusive process in the extremities and the amount of superimposed vasospasm in the collateral circulation   With the history of the case, the physical examination, and the information derived from such a study, the prognosis in a given case can be estimated fairly adequately

SUMMARY AND CONCLUSIONS

Continuous observations of surface temperature, before and during sympathetic ganglionectomy, were made on all four extremities of subjects with Raynaud's disease and thrombo-angiitis obliterans   An electromotive thermometer was used   The difference between these two diseases is well illustrated by this study   General anesthesia alone produced maximal vasodilation in Raynaud's disease and thrombo-angiitis obliterans   Severance of the sympathetic nerves did not cause additional vasodilation   In Raynaud's disease a prompt and uniform vasodilating response was observed in the peripheral vessels of all four extremities, but in thrombo-angiitis obliterans uniform response was absent because the occlusive process in the vessels of all four extremities is never the same in thrombo-angiitis obliterans

The graphic records made with anesthesia at the time of operation serve as a check on preoperative studies, carried out with other vasodilating agents   If satisfactory rises in the surface temperature of the involved extremities are not observed after induction of general anesthesia, it is evident that an additional rise will not develop after interruption of the sympathetic innervation

General anesthesia constitutes a satisfactory method for investigating thermal changes in cases of thrombo-angiitis obliterans, Raynaud's disease, vasospastic scleroderma, and arthritis, prior to and during sympathetic ganglionectomy   Valuable information may be obtained regarding the amount and extent of vasospasm in the vessels of the extremities, particularly in the collateral circulation of subjects with thrombo-angiitis obliterans, and the method gives accurate information regarding the degree of occlusive process in this group of cases   Vessels of patients who are refractory to, or react poorly to, other vasodilating agents will usually undergo maximal vasodilation when general anesthesia is administered

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GLYCOLYSIS IN THE BLOOD OF PATIENTS WITH PERNICIOUS ANEMIA

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Following the discovery by Warburg that tumor cells have quantita tive and qualitative differences from those of normal tissues with regard to their carbohydrate metabolism, many investigators have demonstrated that the method of glycolysis was most satisfactory for the study of the metabolism of the blood cells   Maclean and Weir (1) pointed out the role played by the different blood elements in glycolysis, and showed that the erythrocytes were a vital factor in the sugar consumption of normal blood   Inasmuch as the red blood cells play an important part in the glycolytic activity of normal blood, and since pernicious anemia is a dis ease primarily involving the red cells (both number and type), a study of the glycolysis of the blood in this malady has been undertaken for further investigation of the metabolism of the red cells

METHODS

Blood sugar determinations were made from the blood of nine patients with pernicious anemia before treatment and at the height of the reticulo cyte response, following ventriculin therapy   Red cell, white cell and reticulocyte counts were made on corresponding days

The blood sugar values were calculated according to the micro method of Folin and Wu (2)   All the estimations were made from blood samples obtained before breakfast at 8 A M   The usual procedure was to with draw 10 to 15 cc of blood from the arm vein under sterile precautions, no tourniquet being used in order to avoid stasis   The blood was dis charged into a large test tube, mixed with heparin, and kept in a warm room at a temperature of 37° C   Heparin was used as an anti coagulant as it was shown by Falcon Lesses (3) to have no appreciable effect on the rate of glycolysis

The blood sugar content was determined immediately after the re moval of the specimen from the patient, and then at hourly intervals for 5 hours to estimate the glycolytic activity   The red blood cell and white blood cell counts were made with U S Bureau of Standards pipettes and Neubauer-Levy counting chambers   Reticulocyte estimations were made from brilliant cresyl blue film preparations

TABLE I

Rate of glycolysis in pernicious anemia during relapse

Case number	Red blood cells	White blood cell count	Reticulocyte	Mgm glucose per 100 cc blood — Values after						Rate of glycolysis in mgm glucose per 100 cc blood per hour					
				Initial values	1 hour	2 hours	3 hours	4 hours	5 hours	1st hour	2d hour	3d hour	4th hour	5th hour	Average per hour
	millions per cu mm		*per cent*												
1	1 98	8400	2 0	137	120	109	95	79	70	17	11	14	16	9	13 4
2	2 90	5500	0 5	94	87	80	74	68	58	7	7	6	6	10	7 2
3	1 80	7450	3 1	78	67	62	58	55	47	9	5	4	3	8	5 8
4	2 27	5600	0 7	86	75	65	60	55	53	11	10	5	5	2	6 6
5	1 20	5000	3 7	104	92	91	83	80	79	12	1	8	3	1	5 0
6	1 38	2550	1 8	107	95	90	83	79	75	12	5	7	4	4	6 4
7	1 81	5600	0 8	130	121	108	100	100	93	9	13	8	0	7	7 4
8	83	4900	1 2	120	120	117	115	111	107	0	3	2	4	4	2 8
9	1 27	3650	1 6	115	113	109	107	104	101	2	4	2	3	3	2 8
Average	1 71	5405	1 7	108	99	92	86	81	76	9	7	6	5	5	6 4

DATA

In Table I are presented the results of the glucose determinations of the blood from 9 cases with pernicious anemia during relapse   The average initial glucose value is 108 0 mgm per 100 cc blood   There is a uniform rate of glycolysis averaging about 6 4 mgm per hour, the limits being 5 to 9 mgm   The average rate of glycolysis per hour per million red cells is about 3 7 mgm , which is approximately the same as in normal blood

In Table II are listed the results of the blood sugar determinations of 9 patients with pernicious anemia at the height of the reticulocyte response following ventriculin therapy   The initial average glucose value is 100 mgm per 100 cc blood   The average rate of glycolysis per hour is 10 2 mgm , the extremes being 8 to 13 mgm   The average rate of glycolysis per hour per million red cells is 5 4 mgm   These averages are about 65 per cent more than the glycolytic rate before treatment

Compared to the standard rate of glycolysis of normal blood as established by Schmitz and Glover (4), and Falcon Lesses (3), the glycolytic activity of the blood in pernicious anemia during relapse or early remission is markedly retarded   In Chart 1 is a comparison of the average rate of glycolysis of 9 cases with pernicious anemia during relapse and in early remission with normal glycolytic activity as determined by Schmitz and Glover, and Falcon Lesses

Although the initial average glucose values in the cases of pernicious anemia were slightly elevated, they remained within the upper limits of normal   Many investigators have pointed out the fact that glycolysis proceeds at a faster rate with a high initial glucose concentration   In view of this, then, the rate of glycolysis in pernicious anemia would be even slower by comparison, since the initial glucose values are higher than those of normal blood   It is further noted that the initial values of the blood sugar determinations of patients with pernicious anemia in early remission are less than those of the patients in relapse, yet the average rate of glycolysis in the former cases is about 65 per cent more   In all instances, glycolysis appears to progress at a fairly uniform rate, averaging 15 to 17 mgm per hour in the normal, 5 to 9 mgm per hour in pernicious anemia during relapse (red blood cell counts from 0 83 to 2 90 millions per cu mm), and 8 to 13 mgm per hour in pernicious anemia during early remission (red blood cell counts from 0 80 to 2 90 millions per cu mm)
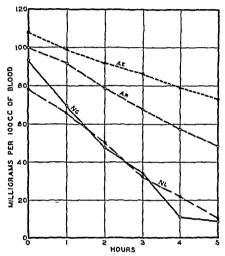
Calculated from figures for normal, the average rate of glycolysis per hour per million red blood cells is about 3 5 mgm   In pernicious anemia, the average rate of glycolysis per hour per million red blood cells is 3 7 mgm   The decreased rate of glycolysis noted in the blood in anemia as compared to that in the normal blood was definitely proportionate to the decreased number of red blood cells   In the cases of pernicious ane-

TABLE II

Rate of glycolysis in pernicious anemia in early remission following ventraculin therapy

Cage number	Red blood cells (millions per cu mm)	White blood cell count	Reticulo cyte (per cent)	Initial values	Values after 1 hour	2 hours	3 hours	4 hours	5 hours	1st hour	2d hour	3d hour	4th hour	5th hour	Average per hour
				Mgm glucose per 100 cc. blood						Rate of glycolysis in mgm glucose per 100 cc blood per hour					
1	2 13	12500	15 5	111	104	72	61	42	31	7	32	11	19	11	16
2	2 90	5000	6 0	85	76	68	59	50	44	9	8	9	9	6	8 2
3	2 25	6950	15 7	81	72	63	55	45	37	9	9	8	10	8	8 8
4	1 80	5500	16 5	92	87	71	51	43	30*	5	16	20	8	13	11 8
5	1 30	4650	22 0	108	89	70	52	40	30*	19	19	18	5	10	15 6
6	2 10	2750	20 0	102	88		76	71	66	14			5	5	8 0
7			12 5	102	93	87	79	68	58	9	6	8	11	10	8 8
8	80	5500	42 0	121	120	108	99	89	80	1	12	9	10	9	8 2
9	1 60	9500	15 7	102	97	91	83	74	66	5	6	8	9	8	7 2
Average	1 86	6544	18 4	100	92	79	68	58	49	8	13	11	10	9	10 2

* Less than 30 mgm

mia in early remission, the blood presents, in addition to a decreased number of red cells, a marked increase in the number and percentage of immature red cells or reticulocytes   The average rate of glycolysis per hour per million red blood cells in this instance is 5 4 mgm   Compared to the rate of glycolysis in normal blood there is a decrease proportional to the decreased number of red cells present   However, in comparison to the rate of glycolysis per hour per million red blood cells, in pernicious

GLYCOLYTIC RATE IN VITRO OF NORMAL AND
PERNICIOUS ANEMIA BLOOD

NG = NORMAL OF SCHMITZ -GLOVER   ------17 CASES
NL = NORMAL OF FALCON-LESSES   --------6 CASES
AE = PERNICIOUS ANEMIA BLOOD BEFORE
                         TREATMENT-9 CASES
AR = PERNICIOUS ANEMIA BLOOD AT HEIGHT
              OF RETICULOCYTE RESPONSE-9 CASES

CHART I

anemia in early remission the glycolytic activity is more rapid than in normal blood   Per unit number of red blood cells (with all factors constant), the only difference between the two is the increased number of reticulocytes   This one factor probably accounts for the accelerated rate of glycolysis which is present in the blood in early remission   As suggested by Barer, Needles and Baldridge (5), this increase in the glycolytic activity may indicate that immature red blood cells have a more active metabolism than adult red blood cells

Five of the patients had higher red cell counts a week after therapy

was instituted, two the same, and one less, yet in all instances the rate of glycolysis was faster with the presence of increased numbers of reticulocytes   In Case 6, the rate of glycolysis was determined before therapy, at the height of the reticulocyte response, and after the immature red cells returned to normal numbers   The glycolytic activity was accelerated when the reticulocytes were present in increased numbers, although the red cell count in this instance was the lowest   This is added evidence that the type of cell present is as important as the number of cells .

According to Glover, Daland, and Schmitz (6), there is a difference of 0 004 mgm per hour in the rate of glycolysis with white cell counts showing a variation of 10,000 per cu mm   In the 9 cases of pernicious anemia, the greatest difference in white cells was 6000 per cu mm   It would seem that the effect of the white blood cells on the rate of glycolysis in pernicious anemia is extremely small, the glycolytic activity being primarily correlated with the number and type of red cells which were present

CONCLUSIONS

1 The average rate of glycolysis per hour in vitro in the blood of 9 cases of pernicious anemia in relapse was 6 4 mgm , the range being 5 to 9 mgm at 37° C

2 The average rate of glycolysis per hour in the blood of 9 cases of pernicious anemia in early remission was 10 2 mgm , the range being 8 to 13 mgm at 37° C

3 The average rate of glycolysis per hour per million red blood cells in blood of normal individuals is 3 5 mgm , in pernicious anemia in relapse 3 7 mgm , in pernicious anemia in early remission 5 4 mgm at 37° C

4 The retarded rate of glycolysis in pernicious anemia is proportional to the red cell decrease

5 With all other factors constant, the increase in the rate of glycolysis per hour per million red cells in pernicious anemia in early remission is associated with the increased number of reticulocytes present

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PLASMA PROTEIN CHANGES AND SUSPENSION STABILITY OF THE BLOOD IN LOBAR PNEUMONIA

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Numerous studies have been made on the relation of fractional blood protein changes to bacterial infection and immunity   The subject is of especial importance in view of the renewed interest regarding the association of immune bodies with the globulins and recent studies in regard to the effects of blood protein changes and plasma viscosity on specific and non specific agglutination reactions (1, 2)   Since the early experiments of Langstein and Mayer (3), it has been generally found that a relative or absolute increase of the globulin fraction and a decrease in the albumin fraction occur during and after various bacterial infections   Glaessner (4) and later Hurwitz and his co workers (5) showed that increases in globulin may occur during the course of immunization as well   They believed that the globulin increase indicated disturbed metabolism and that no direct parallelism existed between the amount of globulin and the degree of immunity   There was no constant relationship between the increased amount of globulin and the antitoxic potency of prepared sera   Similar findings were noted by Schmidt and Tuljtschinskaja (6) and others   Studies on fractional blood protein changes in malaria, typhoid fever and after typhoid vaccination were made by Lloyd and Paul (7)   They observed a marked decrease in the albumin fraction, an increase in the euglobulin and slight or no changes in the total globulin during the acute stage of typhoid fever and malaria   Similar but less marked changes followed vaccination with typhoid bacilli

Many studies have been made on the blood protein changes in lobar pneumonia   Kumpf (8) collected data from 29 reports   It was generally agreed that the total protein was lowered, the fibrinogen and globulin increased and the albumin decreased in amount   No particular attention, however, was given to the speed with which changes in plasma proteins occur after the onset of infection or the time required before the normal equilibrium was regained

Increase of plasma globulin and fibrinogen during infections causes a decrease of the suspension stability of the blood and consequent reduction of the sedimentation time of erythrocytes   The correlation between plasma protein changes and sedimentation time has been demonstrated

repeatedly (9, 10, 11, 12, 13)   In a statistical analysis of the effect of blood protein fractions on the sedimentation rates in a large series of mixed clinical cases, Westergren and his co-workers (12) found a distinct correlation for fibrinogen and globulin and a negative correlation for albumin   Other factors, including hydremia, anemia, changes in cell volume and color index, also influence the suspension stability (14, 15, 16, 17)   Possibly because of these other factors, a number of observers have been unable to establish a constant correlation between fractional protein changes and sedimentation time (18, 19, 20, 21)   In two reports (19, 21) the changes in the amount of fibrinogen, which is known to play the most important role in suspension stability, were not measured

The studies reported in this paper were undertaken to determine the correlation between fractional plasma protein changes, plasma viscosity and the sedimentation time of erythrocytes during and after lobar pneumonia

METHODS

Plasma Protein Determinations (Supervised by Dr Grace Medes) Twenty cc of blood was withdrawn from the arm vein with minimum stasis, and oxalated   Plasma protein fractions were determined by Berglund's modification (22) of Howe's method using sodium sulphate as a salting-out agent   Nitrogen determinations were made by the micro-Kjeldahl technic   The average normal protein values obtained with this method in grams per 100 cc plasma were

Total protein	6 67		
Albumin	4 07		
		fibrinogen	29
		euglobulin	68
Globulin	2 59	pseudoglobulin I	94
		pseudoglobulin II	68

Sedimentation time   At the risk of confusing comparative data, the sedimentation time was observed by employing the usual standard hospital apparatus in place of special tubes   Nine-tenths cc of blood was drawn into a tuberculin syringe (4 mm diameter) containing 0 1 cc of a 10 per cent solution of sodium citrate   The syringe was inverted a number of times to insure thorough mixing, the needle was removed and replaced by a short piece of rubber tubing   The end of the tube was bent back along the barrel of the syringe and fastened with a rubber band   The syringe was placed in a vertical position, stoppered and observed at room temperature   The number of minutes required for the erythrocyte level to reach the 0 6 cc mark was arbitrarily considered to be the sedimentation time   The average normal sedimentation time by this method was 3 hours or more

The Hess viscosimeter was used for the determination of plasma viscosity   According to the technic employed the viscosity of normal plasma was from 1 6 to 1 7

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THE BLOOD IN CASES OF UNEXPLAINED GASTRIC ANACIDITY [1]

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The question of the relationship of gastric anacidity to anemia has recently been the subject of renewed interest and study   Some writers believe that people with unexplained achlorhydria are potential cases of pernicious anemia, subacute combined sclerosis or hypochromic anemia [3] (microcytic, idiopathic, achylic, simple)   Others, however, feel that the view that anacidity bears a causal relationship to these disorders requires further support and that in most cases defective gastric secretion is a harmless variation from the normal

While it must be admitted that many patients with anemia have a gastric anacidity no comparative study, as far as we know, has been made of the blood in comparable groups of people on the one hand with defective and on the other with normal gastric juice, inasmuch as a control of this sort seems fundamental in interpreting the problem we have carried out such observations and they are herewith reported

LITERATURE

Einhorn (1) (1892) demonstrated that anacidity can be present for several years without the development of pernicious anemia   Later (1903) he (2) found some degree of anemia in twelve out of fifteen cases and in four the hemoglobin was below 60 per cent   Three of these patients probably had pernicious anemia

At the International Congress of Medicine in London (1913) Faber (3) proposed that the anemia frequently associated with achylia gastrica, was a secondary phenomenon produced by an absence of gastric secretion Among two hundred and one cases of achylia gastrica, Faber found fifty-nine with a hemoglobin below 80 per cent   Twenty two were examples of Addison s anemia, and thirty seven, of simple anemia   Twenty two of the cases of simple anemia were severe, the hemoglobin being below 65 per cent   Weinberg (4) (1920) states that the blood picture is often

[3] For the purpose of convenience, this condition will be referred to in this paper as hypochromic anemia

abnormal in anacidity, but Alsberg (5) (1921) noted anemia in only one of his seventy cases   Hurst has maintained for many years that the absence of hydrochloric acid from the gastric secretion is the essential predisposing cause of Addison's anemia and subacute combined sclerosis For his conceptions, the reader is referred to his papers which have been collected into a single volume (6)

Hunter (7) (1923) analyzed the findings in sixty patients with anacid-ity most of whom had chronic diarrhea   Seven had a moderate or severe secondary anemia   Faber and Gram (8) (1924) restudied the problem in a new series of ninety cases excluding those with carcinoma, pernicious anemia or tuberculosis   Anemia was defined as a hemoglobin value less than 96 per cent in men and 88 per cent in women   The percentage of anemia was 41   Schneider and Carey (9) (1928) found in fifty-one cases of achlorhydria, seven with a high color index and seven with a secondary anemia   Borgbjaerg and Lottrup (10) (1929) state that 41 per cent of one hundred and thirty-four cases had a secondary anemia, usually of a mild degree, and that several had a color index over one   Lerman, Pierce and Brogan (11) (1932) observed in normal individuals that the red cell count and hemoglobin tended to vary directly with the level of gastric acidity

Many cases have recently been reported, usually under the heading of hypochromic anemia   This disorder is described as an anemia with small erythrocytes and a low color index, occuring in middle aged women who have an anacidity

A number of writers (Witts (12), Waugh (13), McCann and Dye (14), Dameshek (15), Mills (16), Davies (17), and VanderHoof and Davis (18)) believe that this syndrome is a specific disease and that the anacidity is an important etiological factor   However, Bloomfield (19) has critically reviewed the subject and pointed out that various degrees of anemia may be associated with anacidity and that all types of gastric secretion may be associated with anemia

The fact that anacidity may precede pernicious anemia by an interval of from 3 months to 25 or more years, has been reported by at least eight-een authors   Ivy, Morgan and Farrell (20) have been able to collect a total of thirty-six reported cases   In a review of the hereditary aspects of achlorhydria in pernicious anemia, Conner (21) cites thirty-seven authors He reports that among one hundred and fifty-four relatives of one hun-dred and nine patients having pernicious anemia, the percentage of anacid-ity was 25 9, whereas among those of a control group, the percentage was 15 2

Castle (22), (23), (24), (25) has demonstrated that normal human gastric juice can produce by interacting with beef muscle, a substance as effective as liver in promoting blood and clinical improvement in perni-cious anemia   He believes that pernicious anemia is a deficiency disease

brought about by defective digestion of protein, as a result of achylia gastrica   In support of this hypothesis, he reports a patient without anemia and three with hypochromic anemia, all of whom had an anacidity to histamine, but who nevertheless had the necessary hematopoietic stimulating substance present in their gastric juice   Barnett (26) using the same method of biologic assay was unable to confirm his results on two cases without anemia known to have had an anacidity for several years

MATERIAL AND METHODS

The material consisted of twenty six males and twenty-two females with unexplained anacidity as demonstrated by the previously described histamine technique (27)   The anacidity was usually an accidental finding and most of the patients did not have a primary digestive complaint   Patients with carcinoma, pernicious anemia, hyperthyroidism, syphilis, fever, bleeding, or chronic wasting diseases, such as tuberculosis, were excluded   The known duration of the anacidity to histamine varied from 1 month to 4 years and 8 months   The average for the males was 27 months, and for the females, 19 4 months   In practically all of the cases, the family history was negative or no data was obtainable   In one case it was reported that a brother had died of pernicious anemia

The following blood examinations were made red and white cell counts, including a differential, hemoglobin (Sahli) and color index   In order to obtain more detailed data on the condition of the blood fifteen males and ten females were examined by workers specially trained in hematological methods   Red and white cell counts were made with standardized counting chambers and pipettes   In the differential counts of the leukocytes 200 cells were enumerated   Hemoglobin was determined by the oxygen capacity method of Van Slyke and Neill (28) and color indices were calculated on the basis of a correspondence of 5,000,000 corpuscles and 91 per cent hemoglobin equal to 15 6 grams of hemoglobin per 100 cc of blood   Platelets and reticulocytes were counted by a method devised by Dr Harry A Wyckoff of the Stanford Medical School The average diameter of the red corpuscles was measured by the Price Jones technique (29)   The frequency distribution of the corpuscular diameters was determined with an ocular micrometer, measuring 200 corpuscles in freshly dried smears, fixed and stained with Wright's stain Icterus indices and Van den Bergh tests were done in the usual manner

For a control group, a similar study was made of fifteen males and eleven females of about the same age and physical status, who had, however, free acid in their gastric secretion   These subjects were chosen seriatim from a miscellaneous group of hospital patients, and the criteria for selection were the same as for the anacidity group

RESULTS

In Tables 1 and 2 the age, diagnosis and test meal findings [4] are recorded for the control group   Patients with a wide variety of disorders and with every type of gastric secretion were used

TABLE 1

Age, diagnosis and gastric secretory findings in male controls

Case	Age	Clinical diagnosis	Volume	Free acidity	Total acidity
	years		*cc*	*m Eq /L*	*m Eq /L*
1	31	Indigestion, psychoneurosis	60	92	102
2	58	Abdominal pain (unexplained)	23	66	76
3	42	Pneumonoconiosis	21	40	56
4	42	Abdominal pain (unexplained)	23	78	96
5	48	Duodenal ulcer	42	138	142
6	57	Gastric ulcer	50	94	102
7	46	Psychoneurosis	50	65	66
8	32	Psychoneurosis	20	66	78
9	46	Irritable colon	29	100	113
10	28	Psychoneurosis	27	124	130
11	40	Indigestion	17	112	120
12	56	Duodenal ulcer	35	84	99
13	55	Gastric ulcer	36	98	106
14	68	Chronic hepatitis ?	2	25	35
15	46	Indigestion	35	50	58
Average	46 3		31 6	82	92

TABLE 2

Age, diagnosis and gastric secretory findings in female controls

Case	Age	Clinical diagnosis	Volume	Free acidity	Total acidity
	years		*cc*	*m Eq /L*	*m Eq /L*
1	58	Arteriosclerosis and hypertension	42	105	113
2	63	Arteriosclerosis and hypertension	2	25	35
3	36	Duodenal ulcer	55	52	62
4	71	Psychoneurosis	35	91	96
5	41	Psychoneurosis	18	91	101
6	48	Hypertension	2	6	20
7	61	Auricular fibrillation	12	60	70
8	76	Cholelithiasis, diabetes mellitus	16	86	100
9	56	Chronic cholecystitis	52	74	87
10	57	Diabetes mellitus	32	23	43
11	63	Arteriosclerosis, diabetes mellitus	40	104	108
Average	57 2		27 8	65 2	76 8

[4] The highest 10 minute secretory volumes and highest free and total acidity after histamine are alone recorded

In Tables 3, 4, 5 and 6, a detailed report of the blood of the subjects who were especially studied is presented and in Table 7 the findings are summarized   A number of points revealed by those tables will be discussed

Hemoglobin   The hemoglobin in the male anacidity patients ranged between 65 9 and 102 with an average of 87 6 per cent, in the controls between 80 2 and 100 9 with an average of 93 3 per cent   The average grams per cent for the anacidity group was 14 9, and for the control group, 16 07

Hemoglobin in the female anacidity patients ranged between 54 and 95 with an average of 83 9 per cent, in the controls the values lay between 52 2 and 97 9, with an average of 85 8 per cent   The average grams per cent for the anacidities was 14 42, and for the controls 14 68   The following cases had a marked lowering of hemoglobin male anacidity Case 8, female anacidity Case 11, and female control Case 11   Otherwise, the variations were about the same in both series

Red cell counts   There was no striking difference in the red cell counts of the two groups   Male anacidity Cases 1 and 8, female anacidity Case 9, and female control Case 11 had counts definitely below normal

Color index   The majority of the color indices were about 9 or higher Cases 2, 8 and 11 of the female anacidities, and Case 11 of the female controls, had indices below 8   More controls than anacidities had indices above 1 00

White cell count   All of the counts were within the range usually ac cepted for normal people   No anacidity case had a definite leukopenia nor was the differential count unusual in any of the cases

Platelets   These varied considerably in both series, but were consid ered to be within the range of normal

Reticulocytes   Cases 4, 5 and 11 of the female anacidity group, and Case 6 of the male control group, showed a slight reticulocytosis

Icterus index and van den Bergh   A number of individuals among both the "anacidities" and the controls had either an icterus index or Van den Bergh slightly above normal

Price-Jones curve   There was no significant difference as regards the variation in size of red cells between the anacidities or controls

Smear   Reports such as normal smear, slight anisocytosis, moderate polychromasia, etcetera, were not uncommon in either series   The impression of the worker studying the smear did not always correspond to the type of Price-Jones curve recorded, but usually there was a close agreement between the two   Case 11 of the female anacidities and Case 11 of the female controls had definitely abnormal smears

TABLE 3

Blood studies in male control cases

Case*	Hemoglobin	Red blood cells	Color index	White blood cells	Differential			Platelets	Reticulocytes	Van den Bergh		Icterus index	Price Jones				Smear
					Polys	Lympha	Monos			Direct	Indirect		Lower size	Upper size	Apex	Average	
	per cent	10^6		10^3	*per cent*	*per cent*	*per cent*	10^3	*per cent*				*microns*	*microns*	*microns*	*microns*	
1	16 5	5 13	0 93	13 4	56	38	5	359	2 0	—	0 8	9 0	5 62	8 75	7 50	6 75	Slight anisocytosis
2	16 8	4 92	0 99	9 5	70	24	4	510	0 0	—	0 3	6 0	5 62	10 00	7 50	7 40	Normal
3	17 1	5 00	0 99	7 2	54	40	3	340	0 0	—	0 2	5 8	6 25	10 00	7 50	7 77	Normal
4	15 7	4 14	1 10	10 9	57	38	4	368	1 7	—	0 5	7 0	5 62	9 37	7 50	7 63	Slight anisocytosis
5	16 2	4 85	0 98	13 4	59	38	3	528	0 1	—	1 8	15 0	6 25	9 37	7 50	7 73	Normal
6	13 8	4 24	0 95	8 8	63	30	5	487	2 8	—			5 62	9 37	7 50	7 35	Normal
7	14 6	4 90	0 86	9 3	67	22	7	461	0 0	—	0 4	7 5	5 00	8 75	7 50	7 17	Slight anisocytosis
8	14 6	4 35	0 98	4 9	36	55	5	275	0 1	—	0 3	6 3	6 25	8 75	6 87	7 38	Normal
9	16 3	4 67	1 03	10 7	49	46	4	317	0 1	—	0 5	7 6	5 62	9 37	7 50	7 57	Normal
10	17 2	5 04	1 00	9 8	52	35	7	297	0 0	—	0 5	6 0	5 62	9 37	7 50	7 40	Moderate polychromasia
11	15 7	5 05	0 91	6 1	49	45	2	273	0 0	—	0 8	8 1	5 62	8 75	7 50	7 60	Slight microcytosis
12	15 8	4 75	0 97	12 7	56	36	3	502	0 0	—	0 2	6 6	5 00	10 00	6 87	7 77	Normal
13	17 3	5 47	0 93	9 9	51	27	5	443	0 0	—	1 0	8 0	5 00	9 37	8 12	7 19	Normal
14	16 1	4 45	1 07	8 2	51	39	5	134	1 1	—	4 5	15 7	5 00	9 37	6 87	8 06	Moderate anisocytosis
15	17 5	5 00	1 00	8 2	53	41	6	149	0 2	—	0 3	9 7	5 62	9 37	8 12	7 46	Slight anisocytosis
Average	16 1	4 79	0 98	9 5	56	37	5	363	0 54	—	0 8	8 5	5 58	9 33	7 46	7 48	

* Numbers correspond to those of Table 1

TABLE 4

Blood studies in female control cases

Case*	Hemo globin grams per cent	Red blood cells 10⁶	Color Index	White blood cells 10³	Differential			Plate lets 10³	Reticu locytes per cent	Van den Bergh		Ic terus index	Price Jones				Smear
					Polys per cent	Lymphs per cent	Monos per cent			Direct	Indi rect		Lower size mi crons	Upper size mi crons	Apex mi crons	Aver age mi crons	
1	13 8	4 47	0 91	9 8	68	24	3	345	0 0	—	0 5	6 6	5 62	9 37	7 50	7 36	Normal
2	12 6	4 37	0 88	12 6	70	24	4	351	0 1	—	0 3	5 8	5 62	9 37	7 50	7 40	Normal
3	15 3	5 09	0 87	9 2	60	32	5			—	0 8	8 9	4 35	9 37	6 25	6 73	Definite microcytosis
4	16 1	4 40	1 06	9 9	61	26	7	431	1 0	—	1 0	9 5	6 25	9 37	8 12	7 94	Normal
5	16 3	4 75	1 00	9 1	62	32	7	346	0 8	+	0 5	7 0	6 25	9 37	7 50	7 53	Moderate anisocytosis
6	15 1	4 20	1 06	11 3	70	25	2	335	1 4	—	1 0		5 62	9 37	6 87	7 40	Normal
7	15 1	4 04	1 09	9 2	55	29	7	327	0 1	—	0 3	5 7	5 62	11 25	8 12	7 72	Normal
8	15 7	4 65	1 00	14 0	56	32	6	260	0 0	—	0 2	7 8	5 62	9 37	7 50	7 62	Normal
9	15 8	4 49	0 99	8 0	69	15	13	242	0 9	—	0 5	6 9	5 62	10 00	8 12	7 73	Slight anisocytosis
10	16 8	4 22	1 15	7 5	57	40	3	236	1 0	—	0 3	6 5	5 62	8 75	8 12	7 34	Slight anisocytosis
11	8 9	3 68	0 7	8 2	61	33	3	379	0 4	—	0 3	5 0	5 00	8 75	6 25	6 51	Extensive anisocytosis
Average	14 7	4 40	0 98	9 9	63	28	5	296	0 5	—	0 5	6 3	5 56	9 48	7 44	7 39	

* Numbers correspond to those of Table 2

TABLE 5

Age, diagnosis and blood studies in male anacidity cases

Case	Age (years)	Clinical diagnosis	Hemoglobin (grams per cent)	Red blood cells (10³)	Color index	White blood cells (10³)	Differential Polys (per cent)	Differential Lymphs (per cent)	Differential Monos (per cent)	Platelets (10³)	Reticulocytes (per cent)	Van den Bergh Direct	Van den Bergh Indirect	Icterus index	Price-Jones Lower size (mi-crons)	Price-Jones Upper size (mi-crons)	Price-Jones Apex (mi-crons)	Price-Jones Average (mi-crons)	Smear
1	73	Arteriosclerosis, myocarditis	12.2	3.54	1.01	10.6	80	11	9	574	0.3	—	0.8	11.2	5.62	8.75	7.50	7.44	Normal
2	60	Indigestion	15.8	4.92	0.93	13.0	75	18	5	221	0.0	—	0.3	5.6	5.62	9.37	7.50	7.81	Normal
3	69	Adenoma of thyroid	15.7	4.64	0.99	9.8	69	23	5	269	0.6	—	0.3	10.2	5.62	9.37	7.50	7.28	Slight anisocytosis
4	45	Irritable colon acne rosacea	15.5	4.60	0.98	8.8	73	20	1	349	0.4	—	0.8	8.2	5.62	9.37	6.87	7.24	Normal
5	47	Indigestion	16.5	5.10	0.97	7.1	63	25	8	183	0.0	—	0.3	11.0	5.62	10.00	8.12	7.40	Normal
6	46	Epilepsy	17.5	5.24	0.97	9.5	70	27	8	387	0.6	—	0.2	6.1	5.00	10.00	6.87	7.64	Slight macrocytosis
7	44	Chronic arthritis	14.8	4.33	1.00	11.8	65	27	2	473	2.2	—	0.6	7.5	6.25	8.75	7.50	7.43	Slight poikilocytosis
8	61	Arteriosclerosis	11.3	3.40	0.99	8.2	55	21	9	686	0.1	—	0.2	4.8	5.62	9.37	8.12	7.54	Slight anisocytosis
9	60	Arteriosclerosis hypertension	12.3	4.12	0.95	9.7		32	9	151	0.2	—			5.62	10.00		8.31	Slight anisocytosis
10	67	Arteriosclerosis chronic prostatitis	14.0	4.42	0.93	9.1	73	18	6	206	0.6	—	1.0	7.8	6.25	9.37	7.50	7.32	Slight anisocytosis
11	58	Cerebral arteriosclerosis	17.5	4.91	1.03	9.5	61	27	10	400	0.8	—	0.5	9.0	5.62	9.37	7.50	7.58	Definite anisocytosis
12	44	Indigestion	15.7	4.54	1.02	8.5	67	23	8	345	0.3	—	0.5	7.6	5.62	9.37	7.50	7.55	Normal
13	62	Angina pectoris	16.0	4.33	1.01	6.9	54	28	7	338	0.0	—	0.8	9.0	5.00	10.00	7.60	7.40	Slight anisocytosis
14	76	Arteriosclerosis myocarditis	13.4	4.02	0.85	15.6	58	30	8	410	0.0	—	0.2	5.6	5.00	8.75	7.60	7.64	Slight anisocytosis
15	64	Chronic constipation	17.0	4.55	0.90	8.6	55	35	5	242	0.2	—	0.5	7.6	5.00		6.87	6.92	Normal
		Average	14.9	4.55	0.97	9.6	65	24	7	301	0.5	—	0.5	7.4	5.49	9.48	7.41	7.49	

TABLE 6

Age diagnosis and blood studies in female onacidity cases

Case	Age	Clinical diagnosis	Hemoglobin	Red blood cells	Color Index	White blood cells	Differential			Platelets	Reticulocytes	Van den Bergh		Icterus index	Price-Jones				Smear
							Polys.	Lymphs.	Monos.			Dl rect	Indl rect		Lower size	Upper size	Apex	Average	
	years		grams per cent	10^6		10^3	per cent	per cent	per cent	10^3	per cent				mu cross	mu cross	mu cross	mu cross	
1	51	Irritable colon	16.3	5.64	0.84	10.6	72	23	3	983	0.5	—	0.5	6.0	5.62	8.75	6.87	6.80	Slight microcytosis
2	38	No disease	16.0	6.27	0.73	7.9	60	35	3	315	0.1	—	0.5	6.8	5.0	10.62	6.87	7.33	Normal
3	47	Psychoneurosis	14.6	4.70	0.91	8.9	47	47	4	25	1.6	—	0.8	8.8	5.00	8.75	6.87	6.98	Normal
4	55	Irritable colon	14.5	4.61	0.91	6.5	68	22	6	194	4.6	—	0.2	6.0	5.62	10.00	6.87	7.41	Slight anisocytosis
5	37	Obesity hypertension	15.1	5.00	0.93	12.1	65	27	5	343	3.4	—	—	7.3	5.0	9.37	7.50	7.38	Slight anisocytosis
6	28	Psychoneurosis	15.0	5.00	0.90	14.3	77	15	4	440	1.2	—	0	11.4	5.62	9.37	7.50	7.65	Normal
7	65	Indigestion	12.7	4.28	0.87	9.9	43	47	4	248	0.1	—	0.3	5.0	6.25	10.00	7.50	7.72	Normal
8	50	No disease	15.5	5.79	0.76	9.3	69	26	4	139	0.2	—	0.2	7.8	5.62	9.37	6.87	7.09	Moderate anisocytosis
9	55	Abdominal pain (unexplained)	14.8	3.97	1.10	8.4	67	24	7	429	0.4	—	0.5	9.0	5.00	10.00	7.50	7.5	Normal
10	49	Periarteritis nodosa	9.3	4.23	0.64	9.6	67	19	12	516	5.6	—	0.3	9.0	3.75	10.00	7.50	7.05	Marked anisocytosis
Average			14.4	4.93	0.88	9.7	63	30	5	319	1.8	—	0.6	7.3	5.37	9.02	7.19	7.26	

TABLE 7

Summary of average findings in anacidity and control cases

	Hemo-globin	Red blood cells	Color index	White blood cells	Differential			Plate lets	Reticu-locytes	Van den Bergh		Icterus index	Price Jones			
					Polys	Lymphs	Monos			Direct	Indirect		Lower size	Upper size	Apex	Aver-age
	grams per cent	10^6		10^3	per cent	per cent	per cent	10^3	per cent				microns	microns	microns	microns
Male anacidities	14 90	4 55	0 97	9 6	65 4	24 3	6 5	361	0 46	—	0 45	7 4	5 49	9 48	7 41	7 49
Male controls	16 07	4 79	0 98	9 5	55 5	36 9	4 5	363	0 54	—	0 78	8 5	5 58	9 33	7 46	7 48
Female anacidities	14 42	4 93	0 86	9 7	62 5	29 7	5 4	319	1 76	—	0 59	7 3	5 37	9 62	7 19	7 26
Female controls	14 68	4 40	0 98	9 9	62 6	28 4	5 1	296	0 51	—	0 50	6 3	5 56	9 48	7 44	7 39

DISCUSSION

A study of the tables shows that many people with anacidity have subnormal blood counts when comparison is made with standard "normal" or "ideal" values   The point at issue, however, is not this indisputable fact but whether the anacidity bears a causal relationship to the hematological deviations   To settle this point controls are clearly necessary and when a group of people of similar age, sex and condition, *but with normal gastric secretion* is studied by the same methods no significant difference appears   This matter of controls has not, we believe, been properly emphasized in previous work, hence the rather generally accepted idea that anacidity *per se* leads to deficiency of the blood   If, for example, Case 11 of the female control series had had an anacidity she would doubtless have been considered a typical case of "achlorhydric hypochromic anemia" by most writers   A further complication comes from the fact that many of the achlorhydric women with hypochromic anemia reported in the literature had lost blood over long periods of time from uterine hemorrhage, in the present study all patients thought to have abnormal bleeding were eliminated

Whether or not people with anacidity as a class are specially liable to develop pernicious anemia is a debatable question   Certainly there are a few isolated instances on record in which the anacidity has preceded the anemia by many years   However, some of the individuals in this series have had an anacidity to histamine for over four years and others are known to have had an anacidity to other test meals for a longer period of time without any impairment of health   In the male anacidity series, Case 1 was told that he had no acid in his stomach in 1926, Case 8 was told the same thing in 1908, and Case 9 had an anacidity to the fractional gruel meal in 1925

Although anacidity is practically always part of the disease picture of Addisonian anemia, yet there are many gaps in our knowledge of the role of this defect in the production of the disease   Even though the two are intimately related, it is difficult to explain why the absence of free hydrochloric acid is so common in apparently healthy people

CONCLUSIONS

The blood picture in twenty five cases of unexplained gastric anacidity was compared with that of an otherwise similar group of people except that they had an apparently normal gastric secretion   No significant difference was noted between the two groups   No evidence is therefore forthcoming that anacidity *in itself* leads to anemia

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STUDIES ON THE ACTION OF DIURETICS II THE EFFECT OF SALYRGAN UPON THE WATER CONTENT OF THE PLASMA AS MEASURED BY THE REFRACTIVE INDEX

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There are conflicting reports in the literature concerning the effect of the mercurial diuretics upon the water content of the plasma   Saxl and Heilig (1) Bohn (2 3) and Crawford and McIntosh (4) have reported that the concentration of protein in the plasma falls before the onset of novasurol diuresis   Muhling (5), Nonnenbruch (6) and Bleyer (7), on the other hand, found no such evidence of plasma dilution

In a previous publication (8) evidence was presented that salyrgan diuresis is the result of decreased reabsorption of water in the renal tubules It was suggested that this decrease in reabsorption is produced by a depressant action of the drug on the tubular epithelium   The occurrence of hydremia preceding the onset of diuresis would be inconsistent with this hypothesis and would point to a primary extrarenal action, occurring alone or in conjunction with a direct action on the kidney   Carefully controlled studies of the effect of salyrgan upon the refractive index of the plasma have therefore, been carried out   For purposes of comparison similar studies have been made with intravenously administered physiological saline solution

PROCEDURE

The experiments were performed upon unanesthetized female dogs who had gone without food for twelve hours or more   In Control Experiments 1 to 7 inclusive, and in the first seven salyrgan experiments, the animals were permitted to drink water as desired until two hours before the beginning of the experiment   In all other experiments the dogs were allowed no water for five hours preceding the period of observation   During the experiment the dog was tied down on a table   After a period of training the animals would remain in this position for two to three hours without struggling   Urine was collected from the bladder directly into graduated cylinders by an inlying catheter   Blood samples were drawn from the external jugular vein without stasis   Heparin (0 01 cc of a 4 per cent solution to each cc of blood) was used to prevent clotting   In Control Experiments 1, 2, 3, and 4 and in salyrgan Experiments 1 and 2 the blood

samples were from 5 5 to 6 0 cc each   In all other experiments about 1 5
cc of blood were drawn at each venepuncture   Immediately after with-
drawal the blood specimens were centrifuged for two minutes at 1800
r p m , and the refractive index determined on a drop of the plasma in
an Abbe refractometer at room temperature   The temperature seldom
varied as much as one degree centigrade during the course of an experi-
ment   A rise of one degree in the temperature was found to produce a fall
of approximately 0 0001 in the refractive index of the plasma   Changes
in temperature of less than one degree were therefore ignored   On the
few occasions when greater variations than this occurred the refractive
index was corrected correspondingly

<div align="center">RESULTS</div>

<div align="center">(a) Control experiments</div>

The refractive index of the plasma was found to fluctuate considerably
under controlled conditions (Figs 1A and 1B)   Of particular interest
in connection with this investigation are the decreases which occurred in
Control Experiments 3, 4, 5, 6 and 7   In these five experiments the index
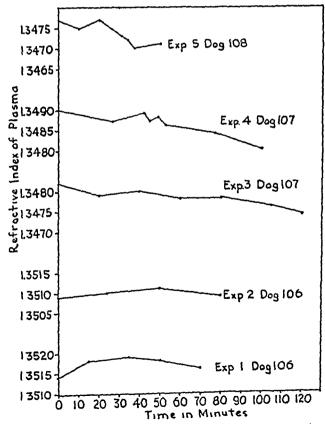

<div align="center">FIG 1A   CONTROL EXPERIMENTS 1, 2, 3, 4 AND 5</div>

In Experiments 1, 2, 3, 5 and 10 the index did not vary more than plus or minus 0 0002 from the control period level   In Experiment 9 a temporary increase of 0 0005 occurred after the administration of salyrgan   The index then returned to and remained at approximately the control period level until diuresis began   In another instance (Experiment 8) a more persistent rise in the index occurred   In the remaining three experiments
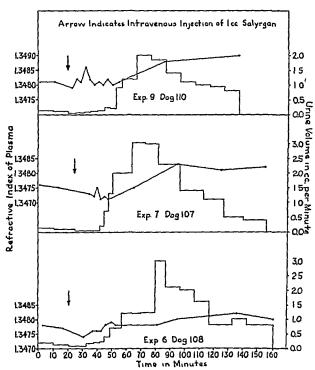

FIG 2B   SALYRGAN EXPERIMENTS 6 7 AND 9

(4 6 and 7) the index fell 0 0006 0 0003 and 0 0004 (0 3 0 15 and 0 2 gram per cent of protein) respectively   Decreases of equal magnitude and occurring within a similar period of time were observed in the control experiments without any subsequent increase in urine volume

In all ten salyrgan experiments the refractive index of the plasma increased gradually during the early part of diuresis   When the index had

a considerable rise occurred   These results may, however, be merely a matter of chance   A rise in the index occurred in Experiment 1, and the index remained fairly constant in Experiment 2, although water was withheld for only two hours in both of these experiments   In the control period of salyrgan Experiment 10, on the other hand, a precipitous drop in the index occurred even though the dog had received no water for five hours

The urine output in all of the control experiments either remained at about the same level or slowly declined during the period of observation The spontaneous decreases in the index of refraction of the plasma were never followed by diuresis

(b) Salyrgan experiments

No consistent change in the refractive index of the plasma was observed before the onset of salyrgan diuresis (see Figures 2A, 2B, and 2C)
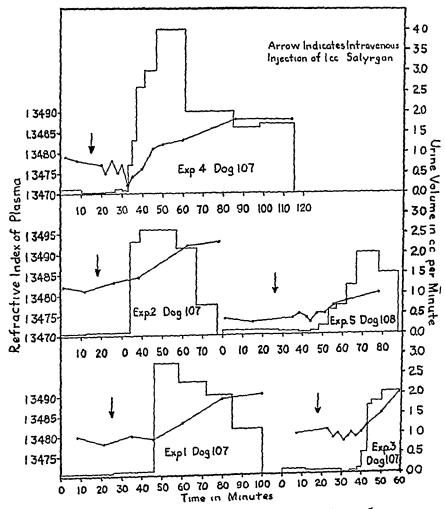

FIG 2A   SALYRGAN EXPERIMENTS 1, 2, 3, 4 AND 5

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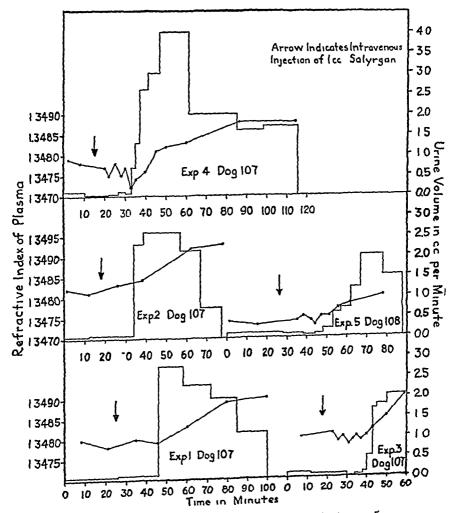

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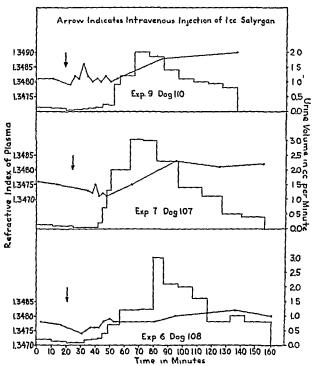

FIG 2B   SALYRGAN EXPERIMENTS 6 7 AND 9

(4, 6 and 7) the index fell 0 0006 0 0003 and 0 0004 (0 3, 0 15 and 0 2 gram per cent of protein) respectively   Decreases of equal magnitude and occurring within a similar period of time were observed in the control experiments without any subsequent increase in urine volume

In all ten salyrgan experiments the refractive index of the plasma increased gradually during the early part of diuresis   When the index had

risen approximately 0 0010 (representing an increase of 0 5 gram per cent in the protein concentration) no further increase occurred

(c) Saline experiments

Varying amounts of 0 9 per cent saline solution were injected intravenously at the rate of 10 cc per minute (see Figures 3A and 3B) In
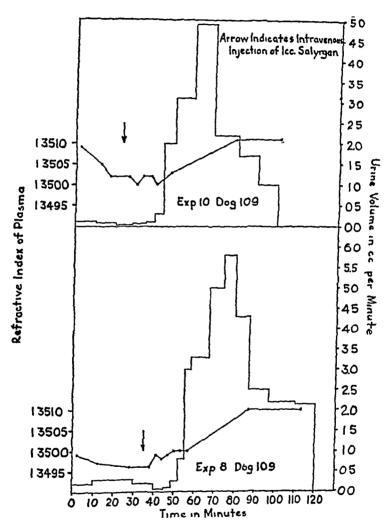

FIG 2C   SALYRGAN EXPERIMENTS 8 AND 10

Experiments 1 and 2 the index of refraction of the plasma fell 0 0006 and 0 0009, respectively, without any increase in the urine volume   In the other four experiments, in which the index was lowered more than 0 0010, diuresis occurred   There was no correlation, however, between the magnitude of the urine volume and the degree to which the index of refraction

was decreased   Only slight diuresis occurred in Experiments 3 and 5 in which the index fell 0 0015 and 0 0023 respectively, whereas the urine output was augmented considerably in Experiments 4 and 6, in which the index dropped 0 0017 and 0 0013, respectively below the control period level

COMMENT

Bohn has suggested that the contradictory reports in the literature concerning the effect of novasurol upon the water content of the plasma are
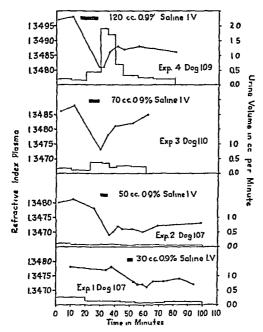

FIG 3A   SALINE EXPERIMENTS 1, 2 3 AND 4

due to the fact that blood samples were taken at different times and that the temporary hydremia was missed by some investigators   In the present experiments samples of blood were taken at approximately three minute intervals during the period between the administration of salyrgan and the onset of diuresis   There is no evidence in these experiments that diuresis is preceded by a mobilization of fluid from the tissue spaces   The refractive index of the plasma showed no consistent change before the onset

of diuresis and such decreases as were observed fell within the limits of variation noted in the control experiments

The argument has been advanced that the kidneys respond so promptly with diuresis when a shift of fluid from the tissues to the blood occurs that it is impossible to demonstrate appreciable dilution of the plasma   The results of the saline experiments do not support such a hypothesis   They demonstrate that the refractive index of the plasma may be lowered markedly by the injection of physiological saline solution without producing sig-
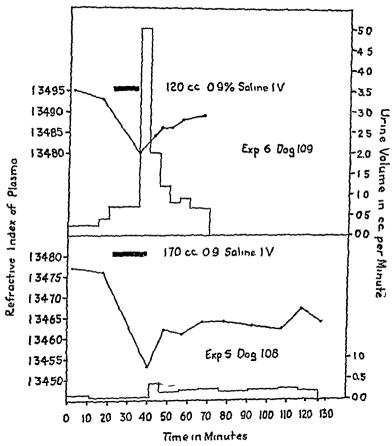

FIG 3B   SALINE EXPERIMENTS 5 AND 6

nificant diuresis   This is additional evidence that the moderate decrease in the refractive index of the plasma observed in some of the salyrgan experiments played no significant rôle in the production of diuresis

The refractive index of the plasma rises gradually during the early part of salyrgan diuresis   When the index has increased approximately 0 0010 no further concentration of the plasma occurs   Apparently sufficient fluid flows into the plasma from the tissue spaces to balance the loss by way of the kidneys

The amount of water lost from the plasma may be calculated roughly from the change in the refractive index Salyrgan Experiment 3 in which a comparatively moderate diuresis occurred may be used as an example Dog 110 weighed 14 kilograms If we estimate the plasma volume as 600 cc and assume a protein concentration of 6 grams per 100 cc, then an increase of 0 5 gram per cent in the protein concentration (a rise of 0 0010 in the refractive index) would mean a loss of approximately 50 cc of water from the plasma The total amount of urine passed from the onset of diuresis to the end of the experiment was 105 cc In Experiment 8 the urine exceeded the calculated loss of water from the plasma by approximately 150 cc

The excess of urine volume over the calculated loss of fluid from the plasma points to an inflow of tissue fluids into the blood The absence of hydremia preceding the onset of diuresis the gradual rise of the refractive index of the plasma during the early part of diuresis and the maintenance of a level after the index has risen approximately 0 0010 indicate that this inflow is secondary to the loss of fluid from the blood by way of the kidneys This secondary withdrawal of fluid from the tissue spaces is probably the mechanism by which the mercurial diuretics remove accumulations of fluid in the subcutaneous tissues and in the peritoneal cavity

SUMMARY

The effect of salyrgan upon the water content of the plasma has been studied by means of the refractometer

There is no evidence in these experiments that salyrgan diuresis is preceded by a mobilization of fluid from the tissue spaces

The results point strongly to a primary direct action of salyrgan on the kidney with a secondary inflow of fluid from the tissue spaces to prevent excessive dehydration of the plasma

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STUDIES IN CONGESTIVE HEART FAILURE XXIIII A CRITICAL STUDY OF METHODS FOR DETERMINING THE CARDIAC OUTPUT IN PATIENTS WITH CARDIAC DISEASE

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The cardiac output of normal persons may be determined by methods the accuracy of which has been adequately demonstrated (4) In patients with congestive heart failure on the other hand so many factors intervene that the validity of a method applicable to normal individuals may no longer be assumed Hitherto comparatively little work has been done in testing the applicability of various cardiac output methods to patients with heart disease although data obtained by such methods may be subject to large errors

In the present investigation we have examined the accuracy of three methods of measuring the cardiac output and ascertained the extent of their applicability to subjects with congestive heart failure The Burwell and Robinson procedure (3), the venous plateau method (7), and the acetylene procedure (4) seemed to us to offer the greatest probability of proving applicable in heart disease The method of Burwell and Robinson depends upon the equilibration of the patient's blood with a gas mixture obtained by repeated rebreathings From a comparison of the oxygen content of this blood with that of a sample of arterial blood the arteriovenous difference is derived This method assumes that the same physicochemical equilibrium occurs between the blood and the gas obtained by the rebreathing procedure when shaken in a tonometer as occurs across the pulmonary epithelium during rebreathing Obviously this physiological equilibration occurs only slowly and may therefore lead to illusory constancy in any case in which pulmonary conditions inhibit (without totally preventing) normal oxygenation of the blood in the lungs Since such conditions are probably of frequent occurrence in cardiac disease, Burwell and Robinson, realizing these possible sources of error, limited their observations to individuals with normal lungs

In order to avoid the source of error in the Burwell Robinson procedure Harrison, Friedman and Clark (7) modified it Their venous plateau method depends upon the use of the lungs for the final equilibration

instead of a glass tonometer as in the Burwell and Robinson procedure
The patient rebreathes the final gas mixture, and during the rebreathing,
samples of "mixed venous" blood are drawn from the femoral artery

The acetylene procedure (4) is founded upon a principle entirely dif-
ferent from that of these two methods   It depends essentially upon meas-
uring the rate of absorption of a foreign gas across the pulmonary epi-
thelium

Experiments demonstrating the sources of error occurring in these
methods will be presented and the criteria which must be applied for demon-
strating their validity in a given patient will be defined   When these
criteria are satisfied accurate results may be obtained

The attainment of homogeneous mixture in a lung-bag system in patients
with congestive heart failure

The measurement of the cardiac output in patients with congestive
heart failure is rendered difficult in all methods based upon equilibration
of gas mixtures accross the lungs by essentially two factors   The first of
these is the change in the mechanical conditions in the lungs which makes
it difficult to obtain samples of the gas mixtures which are in true equilib-
rium with the blood   A second factor is the involvement of the alveoli
in a pathological process which prevents the establishment of true equilib-
rium (in a physicochemical sense) between the alveolar gases and the
blood

A prominent feature in patients with heart disease is the marked pul-
monary alteration which mechanically interferes with any rebreathing
procedure   The exact nature of the pulmonary lesions need not be de-
scribed here, suffice it to say that loss of elasticity of the alveolar walls,
congestion of the lungs, swelling of the pulmonary epithelium, the presence
of edema in the alveoli and bronchioles, and, in some cases, emphysema,
combine to increase the residual air and render difficult the attainment
of a homogeneous mixture when a patient rebreathes a given gas mixture
from a bag   If homogeneity of the contents of the lungs and of the bag
is not attained it is obviously impossible to obtain a sample of gas from the
bag which is representative of that in equilibrium with the blood

The Burwell-Robinson, venous plateau and acetylene procedures are
all dependent upon attaining complete mixture in a lung-bag system   It
was, therefore, necessary to determine the length of time required for
obtaining a homogeneous mixture in this system   For this purpose the
method of Lundsgaard and Schierbeck (9), used previously by Grollman
and Marshall (5), was utilized   The subject rebreathes a mixture of
hydrogen in air under the same conditions as in the desired experimental
procedure   By analyzing samples of the gas withdrawn simultaneously
from the bag and from the mouth one can obtain an indication of the
degree of homogeneity in the lung-bag system   The time required is

about fifteen seconds in normal individuals but is longer in subjects with abnormal lungs

There exists no definite and universally accepted nomenclature for describing succinctly the clinical condition of a given patient. The term " decompensation," for example may have a different denotation for different observers   We have included therefore in Table 1 data bearing on the clinical condition of the patients whom we have studied   Comparison of a result in the experimental tables with the clinical description of Table 1 will allow the reader to judge roughly concerning the conclusions to be drawn from the present study

In Table 2 are given the results obtained in a series of patients showing various degrees of congestive heart failure as defined by the clinical data of Table 1   The patients rebreathed a mixture of hydrogen in air as is customary in measuring the cardiac output by the acetylene method   The volume of gas in the bag (Table 2, column 2) was that which could be inspired without difficulty by the patient (4)   The number of respirations and the time after beginning rebreathing when collection of the samples was made are given in columns 3 and 4

Since the respiratory quotient is less than unity during rebreathing (due to accumulation of carbon dioxide in the bag which prevents the usual loss of carbon dioxide from the mixed venous blood in the lungs) the hydrogen content of the sample obtained from the mouth is greater than that expired into the bag when homogeneous mixture has been attained in the lung bag system   A positive difference in the fifth column of Table 2 indicates therefore that mixture has occurred under the conditions of the rebreathing

The data in Table 2 demonstrate the greater length of time necessary in patients with heart failure than in normal individuals for attaining homogeneous mixture between the air in the lungs and a gaseous mixture contained in a bag   Whereas normal subjects seldom require more than fifteen seconds, the subjects in Table 2 required eighteen seconds in two cases, twenty seconds in three cases, and twenty-five seconds or more in the remaining three   The application of these results to the problem of measuring the cardiac output in such cases will be given later

Equilibration of gases across the pulmonary epithelium

The second possible source of error, which must be considered before applying to persons with congestive heart failure a method applicable to normal subjects, is the possible alteration of diffusion through the pulmonary epithelium due to pathological changes   If a change occurs in the lung which diminishes the rate at which a gas can pass into the blood from the alveolar spaces, physicochemical equilibrium may not occur and the method based upon the assumption that it does becomes inapplicable Although much has been said concerning this possibility in studying cases

TABLE 1

Clinical data on subjects with organic cardiac disease studied in this investigation

Subject	Age	Sex	Race	Height	Edema free weight	Surface area	Type of cardiac disease	Degree of cardiac failure						Therapy being administered
								Degree of dyspnea		Vital capacity	Degree of edema	Degree of cardiac enlargement	Râles in lungs	
								On exertion	At rest					
	years			inches	pounds	square meters				liters				
P N	59	M	Negro	67 5	158	1 82	Arteriosclerosis, hypertension	+	0	3 4	0	++	0	None
I M	28	F	White	66 5	121	1 62	Mitral stenosis and insufficiency, auricular fibrillation	+++	+	2 1	+	++	+	Digitalis Diuretics
E G	44	M	Negro	66 5	127	1 62	Syphilitic aortic insufficiency	+++	±	2 7	0	+++	±	Digitalis
F B	43	M	Negro	67 2	135	1 62	Syphilitic aortic insufficiency	+++	++	2 5	++	+++	+	Digitalis Diuretics
U R	37	M	Negro	68 2	132	1 70	Mitral stenosis, auricular fibrillation	+	0	2 9	0	+	0	Digitalis
P F	41	M	Negro	66 7	132	1 66	Syphilitic aortic insufficiency	++	±	2 8	±	+++	±	Digitalis Diuretics
E B	54	M	Negro	67 2	135	1 69	Syphilitic aortic insufficiency	++	0	3 2	±	++	0	Digitalis
W C	69	M	White	68 5	132	1 70	Hypertension, arteriosclerosis, emphysema	++	+	2 4	+	+++	++	Digitalis Diuretics
M Y	74	M	White	66 0	105	1 51	Arteriosclerosis, emphysema	+	±	2 6	++	++	+	Digitalis
M S	39	F	White	66 0	126 5	1 62	Syphilitic aortic insufficiency	+	0	3 1	0	++	+	Digitalis

TABLE 2

The time necessary for attaining homogeneous mixture in a lung bag system

Subject	Volume in bag	Number of respirations	Duration of rebreathing	Difference between hydrogen concentration in alveolar sample and in bag	Time necessary for attaining homogeneous mixture
	liters		*seconds*	*per cent of hydrogen*	*seconds*
P N	1 8	7	18	0 00	20
		10	20	+0 05	
		11	21	+0 03	
		13	23	+0 03	
I M	1 2	7	17	−0 03	18
		6	18	+0 02	
		8	22	+0 08	
		8	25	+0 01	
E G	1 5	7	16	−0 15	20
		7	18	−0 00	
		7	21	+0 01	
		9	25	+0 06	
F B	1 5	9	18	−0 09	25
		10	20	−0 02	
		12	23	−0 05	
		13	26	+0 05	
U R	1 5	8	17	−0 06	18
		9	18	+0 00	
		10	20	+0 01	
P F	1 2	9	19	−0 10	26
		8	23	−0 12	
		8	26	0 00	
		9	28	0 00	
E B	1 3	7	16	−0 04	20
		8	19	0 00	
		10	21	+0 01	
		10	23	+0 01	
W C	1 2	15	50	−0 10	Incomplete mixture

of heart failure, it is ordinarily relatively less important than the factor of inadequate mixture, which has just been discussed

The influence of a pathological condition on diffusion through the lung can be obtained from an analysis of the degree of oxygen saturation of the arterial blood   The fact that significant anoxemia occurs only in severe cases of congestive heart failure is an indication that this is a result only of extreme modifications from normal

It is necessary to consider the possibility of failure to obtain a true equilibrium in cases in which oxygen deficiency of the arterial blood exists

In the "oxygen Fick" methods in which it is necessary to secure equilibrium between the gas in the blood and that in a bag, the difference in tension between the two may not be great   Nevertheless any impediment to the passage of oxygen across the pulmonary epithelium may lead to erroneous results   It was this possibility which led Burwell and Robinson to apply their method to normal individuals only

One of the fundamental assumptions of the acetylene method is the occurrence of true physicochemical equilibrium between this gas in the alveoli and in the blood   That equilibrium occurs in persons with normal circulatory systems and even in some cases of decompensated heart disease was demonstrated by Baumann and Grollman (2) and by Grollman, Proger, and Dennig (6)   In one case (senile emphysema with chronic congestive failure) the former authors failed to observe its occurrence This failure was attributed to an inability to obtain mixture in the lung-bag system and was therefore apparent and not real   Unless one obtains an actual alveolar sample, one cannot judge whether equilibrium has occurred in the lungs by comparison of the gas obtained with a sample of arterial blood   So far as measurement of the cardiac output is concerned, the question is purely academic whether equilibrium has occurred or whether failure to attain it is due to failure to obtain a true alveolar sample   Regardless of the cause, the method is inapplicable in such a case

In Figures 1, 2, 3, and 4 are reproduced typical experiments demonstrating the relation of the concentrations of acetylene in the blood and in the gas mixtures rebreathed   Two patients (Figs 1 and 2) rebreathed an acetylene-air mixture from a rubber bag as is usual in measuring the cardiac output   At certain intervals samples of arterial blood and a gas sample from the bag were collected and their acetylene contents determined as described by Grollman, Proger, and Dennig (6)   It will be noted in
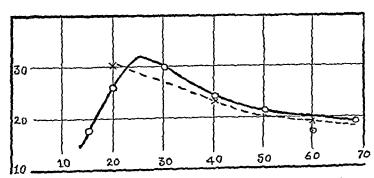

FIG 1   A COMPARISON OF THF ACETYLENE CONTENT OF THE ALVEOLAR AIR AND THE ARTERIAL BLOOD WHILE REBREATHING FROM A RUBBER BAG   SUBJECT E G (SEE TABLE 1)

Ordinates represent the acetylene tension in millimeters of mercury, the abscissae represent the time in seconds after beginning the rebreathing
        ◯ Blood samples   ✕ Gas samples
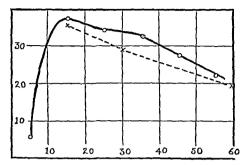
FIG 2   A COMPARISON OF THE ACETYLENE CONTENT OF THE ALVEOLAR AIR
AND THF ARTERIAL BLOOD WHILE REBREATHING FROM A RUBBER BAG   SUB
JECT J M (SEE TABLE 1)

Ordinates represent the acetylene tension in millimeters of mercury, abscis
sae the time in seconds after beginning the rebreathing

O Blood samples   X Gas samples

Figure 1 that there is complete concordance between the amounts of acety-
lene in the blood and in the bag at twenty seconds after beginning the
rebreathing and thereafter   Since this subject could not mix in a period
of time less than twenty seconds as seen in Table 2, the low content of
acetylene in the samples of blood collected at fifteen and twenty seconds
is to be attributed not to failure to attain equilibrium between the acetylene
of the alveoli and the blood, but to the fact that it required twenty seconds
to establish a uniform concentration of gas in the lung bag system   The
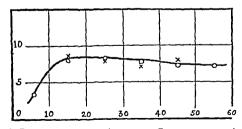remainder of the curve is evidence against the existence in this patient of

FIG 3   A COMPARISON OF THE ACFTYLENE CONTENT OF THE ALVEOLAR AIR
AND THE ARTERIAL BLOOD WHILE REBREATHING FROM A SPIROMETER   SUB
JECT I M (SEE TABLE 1)

Ordinates represent the acetylene tension in millimeters of mercury abscis
sae the time in seconds after beginning the breathing

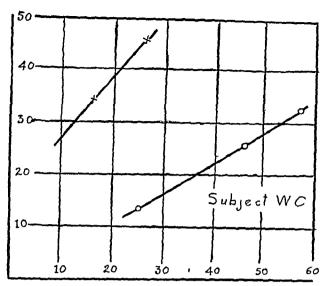O Blood samples   X Gas samples

FIG 4 A COMPARISON OF THE ACETYLENE CONTENT OF THE "ALVEOLAR" AIR AND THE ARTERIAL BLOOD WHILE BREATHING FROM A SPIROMETER SUBJECT W C (SEE TABLE 1)

Ordinates represent the acetylene tension in millimeters of mercury, abscissae, the time in seconds after beginning the breathing

  O Blood samples   X Gas samples

an impediment to the passage of acetylene across the pulmonary epithelium which might interfere with the attainment of equilibrium between the gas in the lungs and in the blood

The curves of Figure 2 were obtained similarly to those of Figure 1 At five seconds, the acetylene concentration in the blood was very low, for insufficient time had elapsed since beginning of rebreathing to allow entrance into the alveoli of sufficient amounts of acetylene   After fifteen seconds mixture in the lung-bag system occurred so that the acetylene content of the blood and of the alveoli was parallel   The concentrations in the blood were higher, however, due to the fact that it requires five to ten seconds for the blood to travel from the lungs to the peripheral artery from which it is drawn   If one allows for this (2, 6), there results an identity of the curves of the concentrations in the alveoli and in the blood which demonstrates the existence of true physicochemical equilibrium In Figure 1, there is also a tendency for the acetylene concentrations to be higher in the blood than in the alveoli but in this case the more gradual slope of the curve prevents this from manifesting itself prominently in the samples collected after forty seconds

The studies of Figures 1 and 2 as well as previous ones of Grollman and his coworkers require the time necessary for the passage of the blood from the lungs to the peripheral artery to be taken into consideration As is evident from the curves of Figures 1 and 2 and from the results of Baumann and Grollman (2), this possible source of error is negligible

being only one or two per cent   This smallness results from the relatively
small drop in acetylene concentration which is taking place in the bag (Figs
1 and 2)   The assumption involved need not be made, however, if a con-
stant alveolar concentration is maintained   This is accomplished by having
the subject inspire at a constant rate from a spirometer containing an
acetylene air mixture   At given intervals forced expirations are made of
which alveolar samples are collected   The results of such an experiment
on I M are given in Figure 3   The concentrations of acetylene in the
alveoli and in the blood lie on a uniform curve after fifteen seconds the
time necessary for substituting the gas mixture for the air in the lungs

Patients I M and E G were both cases of congestive heart failure
(Table 1) and in them apparently the transport of acetylene across the
lungs was sufficiently rapid to ensure equilibrium, despite the fact that
there was a slight degree of arterial anoxemia in both subjects   The
greater ease of attaining equilibrium between the blood and alveoli in the
case of acetylene in comparison with oxygen, is what one might predict on
strictly physical grounds   Indeed, it may be said (in view of the experi-
ments just cited and those previously reported) that it is improbable that
interference with diffusion in the lung is ever the limiting factor of the
acetylene method   Because of its high solubility and its small molecular
weight the tension of acetylene in the blood is the same as that in the alveoli
to which this blood is exposed   But if conditions exist in which part of
the lungs are fibrosed, consolidated collapsed or so markedly edematous
as to prevent its entrance and, therefore, the passage of acetylene into
the blood, the acetylene method cannot measure the blood flowing through
these portions of lungs

One encounters subjects in whom analysis of the acetylene content of
the blood shows a smaller concentration than is present in the supposed
" alveolar " air   An example is shown in Figure 4 which illustrates the
lack of relation between the acetylene contents of the blood and of the
" alveolar " samples   In another similar experiment the same patient in-
spired a mixture of gas, the acetylene tension of which was 54 4 mm , but
whose arterial blood after breathing 55 seconds contained only 29 4 mm
Mixing as tested on this patient (W C   Table 2) was incomplete even
after 50 seconds rebreathing   The discordance in the acetylene content
of " lung air " and blood as observed in this patient cannot be attributed
therefore to any impermeability of the pulmonary epithelium but is due to
an inability to obtain a true alveolar sample

The Burwell Robinson procedure

On the basis of the experiments already cited we can define the condi-
tions under which a given cardiac output method based on respiratory
maneuvres is applicable and the methods whereby its applicability can be
tested

The Burwell-Robinson procedure consists essentially in rebreathing repeatedly from a bag in order to obtain a gas in equilibrium with the mixed venous blood   Not only must mixture in the lung-bag system be ensured but sufficient time must be allowed thereafter for the blood to alter the composition of the gas in the bag   The circulation time cannot be exceeded and hence one is limited to a period of time about thirty seconds (in cases of congestive heart disease) in which to carry out the rebreathing The process can be carried out repeatedly, however, and in this way one may in normal individuals finally obtain a mixture in true equilibrium with the mixed venous blood   Unfortunately, as has previously been indicated (4), constancy of the final mixture cannot (as has often been done in the past) be taken as a proof of equilibration   A patient with a large volume of residual air can replace the air in his lungs partially during the preliminary rebreathing and dilute the gas in the bag to a certain extent only This was the source of error in a number of earlier methods

TABLE 3

The oxygen content of two bags during alternate rebreathings
Subject P F   (Table 1)

Number of rebreathings	Oxygen content	
	Bag 1	Bag 2
	per cent	*per cent*
4		3 76
5	4 95	3 70
6	4 87	
8	4 40	
9	4 17	
11	⊦ ⸱	3 85
12	4 15	

The experiment cited in Table 3 demonstrates how delusive constancy of the composition of the bag mixture may be   The patient in this experiment (P F , Table 1) rebreathed alternatively from bags 1 and 2 as in the Burwell-Robinson method, the bags initially having contained mixtures of different oxygen contents   After nine rebreathings the oxygen content of bag 1 was constant and equal to 4 16 per cent   Bag 2, on the other hand, was little affected after the fourth rebreathing and had an oxygen content even after the eleventh rebreathing of only 3 85 per cent   Both of these bags could obviously not be in equilibrium with the mixed venous blood and hence the supposition of their equilibration with the patient's blood might lead to an erroneous result   It must be remembered also that due to the steepness of the oxygen dissociation curve of blood at the degree of oxygen saturation of the mixed venous blood even slight differences in oxygen tension cause a considerable difference in the oxygen content from which, in turn, is calculated the cardiac output

The extent to which the source of error just described may invalidate attempts to obtain a gas mixture in equilibrium with the mixed venous blood is further demonstrated in Table 4   In this experiment the patient,

TABLE 4

Changes in the oxygen content of the bag during rebreathings in a patient with congestive heart failure (E G   Table I)

Experi ment number	Number of wash breaths	Time for washing	Sample 1		Sample 2		Sample 3	
			Time of collection	Oxygen content	Time of collection	Oxygen content	Time of collection	Oxygen content
		seconds	*seconds*	*per cent*	*seconds*	*per cent*	*seconds*	*per cent*
1	1	5	20	4 27	24	4 27	28	4 19
2	2	5	20	3 99	25	3 99	30	3 91
3	3	7	27	3 23	30	3 30	34	3 21
4	4	7	23	1 41			28	3 56
5	4	9	29	3 38	34	3 28	38	3 34

E G , rebreathed from a bag containing 1 2 liter of nitrogen   Samples were taken at various times during rebreathing   The number of the preliminary " wash " breaths were varied (column 1) in order to vary the oxygen contents of the lung bag system in the different experiments   The results demonstrate the marked effect which preliminary washings have on the final composition of the gas in the bag   This was observed also by Burwell and Robinson (3)   The results also demonstrate the extremely small changes in oxygen content of the bag which can occur during the breathing of mixtures varying as much as one per cent in composition The rise in oxygen content (in the 3d experiment) and the absence of a decrease (in the first and second experiments) of the oxygen content between the collections of the first and second samples are due to inadequate mixture in the lung-bag system   The times in the 5th 7th and 9th columns refer to that which elapsed after beginning the washing   Actual mixture occurs only after the beginning of rebreathing and hence fifteen seconds (in the first and second experiment), or sixteen seconds (in the 4th experiment), do not suffice for attaining homogeneous mixture in the lung bag system at the time of collection of the first sample from the bag

In order to be certain that the value of a given experiment is not destroyed by the illusive constancy described, it is necessary to approach the final equilibrium with various oxygen contents in the bag   After preliminary rebreathing the patient should rebreathe a given mixture and two samples should be taken after adequate mixture has been attained and before recirculation has begun   It may be advisable to measure the oxygen content with an accuracy greater than ± 0 01 per cent in order to detect small changes such as are masked in Table 4 in which the results are accurate only to about ± 0 05 per cent

Another procedure which may possibly avoid the source of error under discussion is one which was employed by Burwell and Robinson Their subjects rebreathed alternately into two bags, the one having an oxygen tension below and the other an oxygen tension above that of the mixed venous blood   In their normal subjects the oxygen tension of the two bags became identical   We have verified their observations in several patients with cardiac disease, but have found others, especially cases of luetic aortic insufficiency, in whom the final values differed markedly as in Table 3 Obviously the results obtained by this method, in patients with heart failure, are open to grave errors unless one ensures the absence of all the vitiating factors just described   Other so-called " Fick " oxygen or carbon dioxide methods are even more liable to error in such patients

The venous plateau method

In applying this method one must consider the possible sources of error outlined in connection with the Burwell-Robinson method in obtaining the gas mixture used in the final rebreathing

In drawing blood from the femoral artery certain precautions must, however, also be observed   In the first place ample time must elapse to allow mixture between the gas in the bag and in the lungs to take place during the final rebreathing   Samples of blood must be drawn either before or within ten seconds after the patient stops rebreathing, otherwise mixture of partially aerated blood with mixed venous blood may give a plateau which is not representative of the true mixed venous blood   Nor can the patient continue to rebreathe after the completion of a circulation time unless the composition of the rebreathed gas be maintained constant, for example, by allowing the patient to breathe from a spirometer containing gas identical in composition with that in the bag after the final rebreathing

Despite the theoretical applicability of this method for permitting the prolongation of the rebreathing time, it is impracticable because anoxemia (particularly in patients with congestive heart disease) causes extreme discomfort and may initiate an attack of cardiac pain in persons with coronary disease or aortic insufficiency   Anoxemia may in fact cause marked reduction in cardiac output with consequent prolongation of the circulation time in diseased hearts   Blood samples taken more than five to ten seconds after cessation of the rebreathing may therefore give a plateau which in no way represents that of the mixed venous blood   The plateau corresponding to the mixed venous blood, to be reliable, must occur in the blood of the femoral artery about ten seconds after adequate mixture in the lung-bag system has occurred   Before this time the mixed venous blood will lose oxygen if the patient has been " overwashed " in the preliminary " wash breaths " or will take it up if this washing has been insufficient Only after homogeneous mixture has occurred does the blood reflect the

composition of the gas mixture in the bag   Correlation of the time at
which an oxygen plateau in the blood occurs with that of the stage of re-
breathing is, therefore, of great importance

A more detailed discussion of the possible sources of error in the Bur-
well-Robinson and venous plateau methods will be given in a future publi-
cation (7)

The acetylene method

The validity of the underlying assumptions of the acetylene method has
been described in detail elsewhere (4) and need not be repeated   In ap
plying the method to cases with congestive heart failure one must consider
the three fundamental requirements of the method   (1) Do the samples
taken for analysis represent the gas which is in equilibrium with the blood
traversing the lungs?   (2) Does the gas in the alveolar air enter into
equilibrium with the blood?   (3) Is the rebreathing procedure completed
before the time of a complete circulation of the blood?

As shown in Table 2 it is necessary to prolong the preliminary rebreath
ing of the acetylene mixture longer in cases of congestive heart failure than
in normal individuals before taking the first sample   If the sample is
taken before mixture occurs the resultant cardiac output will be false and
may be as much as fifty per cent greater or less than the true value   The
question of attaining equilibrium across the pulmonary epithelium as al
ready stated seldom, if ever, is involved in applying the acetylene method
Care must be taken, however, not to continue rebreathing beyond a circula
tion time, otherwise the estimate of the cardiac output will be too low

In applying the acetylene method in congestive heart failure how can
its applicability be tested without undertaking the laborious work of deter-
mining the various factors just outlined?   Taking three samples for analy-
sis instead of two suffices for this purpose   The apparatus previously de-
scribed (4) has been altered to provide another capillary tube which
permits collection of the third sample   In cases of congestive heart disease
the samples are taken preferably at 20, 25, and 30 seconds after beginning
the rebreathing   The samples taken at 20 and 25 seconds are used to cal
culate one value and those taken at 25 and 30 are used for another   If
these agree within the limits of the experimental technical errors one is
assured that the assumptions underlying the applicability of the method are
valid and that the result is accurate   In all measurements this procedure,
involving as it does little extra work, is suggested even in cases in which
the applicability of the method can be taken for granted

In Figure 5 is reproduced an experiment illustrating these points   The
patient (P N , cf Table 1) could bring mixture about, as shown in Table
2, in twenty seconds   Samples collected at 18 and 23 seconds give an
apparent cardiac output of 4 25 liters, a value too high because the first
sample was taken at 18 seconds when mixture was not quite complete and,

had, therefore, a higher content of acetylene than was present in the alveoli and in equilibrium with the blood   When samples were taken at 20 and 25 seconds and at 25 and 30 seconds, values were obtained which agreed within the limits of the accuracy of the analytical methods employed   The average of these values (3 8 liters) was the correct cardiac output of this patient under the condition of the experiment   When the samples were taken at 30 and 35 seconds, the estimation was too low (3 1 liters), because blood containing acetylene had returned to the lungs   In the cases of congestive heart failure which we have studied, recirculation was evident after 30 seconds   This circulation time as observed in these pathological cases is longer than that observed (4) in normal persons due to the greater velocity of the normal blood flow [1]
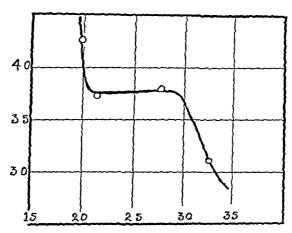

Fig 5   The Effect of Varying the Time of Sampling on the Apparent Cardiac Output as Obtained by the Acetylene Method   Subject P N (not basal)

The ordinates represent the apparent cardiac output in liters per minute, the abscissae the average time of collection of the two samples used in determining the cardiac output   The plateau represents the true cardiac output

Comparison of results by the different methods

The diversity in principles upon which the acetylene method (foreign gas principle) and the Burwell-Robinson and venous plateau methods (oxygen Fick principle) are based gives importance to a comparison of results obtained by these methods when applied to the same patient   In a previous paper, Harrison, Friedman and Clark (7) have shown the excellent

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[1] All discussions of the circulation time in the present paper refer obviously only to man   It is well known (4) that the relative circulatory rates in the smaller mammals is greater than it is in man   Hence it is not surprising that Starr and Collins (Am J Physiol, 1933, civ, 650) should have found an appreciable return of blood to the heart in 15 seconds in dogs   Their conclusions are obviously inapplicable to man

agreement obtained by the various methods in normal subjects. This may be taken as added evidence for the accuracy with which the cardiac output in normal subjects may be measured by these methods. In Table 5 are

TABLE 5

A comparison of results obtained by the Burwell Robinson venous plateau and acetylene methods as applied to patients with congestive heart failure

Subject	Oxygen consumption	Acetylene method		Burwell Robinson method		Venous plateau method	
		Arterio-venous oxygen difference	Cardiac output	Arterio-venous oxygen difference	Cardiac output	Arterio-venous oxygen difference	Cardiac output
	cc. per minute	*cc. per liter*	*liters per minute*	*cc. per liter*	*liters per minute*	*cc. per liter*	*liters per minute*
U. R.	198	90	2.2	94	2.1	90	2.2
M. Y.	170	50	3.4	50	3.4	50	3.4
E. B.	233	71	3.3	61	3.8	68	3.4
M. S.	215	65	3.3	60	3.5	67	3.2
P. F.	212	60	3.6	57	3.7	62	3.4
E. G.	263	81	3.2				
P. N.	258	69	3.7				
I. M.	180	84	2.1				

given a series of results obtained in patients with congestive heart disease. The procedures were performed at one sitting. The results agree within the limits of the experimental errors involved and add further evidence of the validity of the theoretical considerations now discussed and the accuracy of the results obtained.

Relatively few values of the cardiac output in congestive heart failure are to be found in the literature in which the necessary precautions have been taken to demonstrate the applicability of the method to the patient under investigation. In view of the small number of cases which we have studied (Table 5) no final conclusion concerning the cardiac output in cardiac disease seems warranted. Of the eight patients cited in Table 5, the cardiac index (4) is reduced in five and within normal limits in the remainder. This does not necessarily imply, however, that the blood supply to the tissues in these cases is adequate, for under the disturbed conditions in the body, particularly in the presence of edema (8), the normal blood supply may still be inadequate for proper supply of the metabolic requirements of the tissues. During exertion, moreover the cardiac output in these patients may be markedly reduced from the normal (1). We have not yet found patients with congestive failure and abnormally great cardiac output.

Choice of methods

As to the relative merits of using one or another of the methods studied, the laboriousness of the Burwell-Robinson and venous plateau methods

both to the patient and to the investigator renders them inferior to the relatively simple acetylene procedure   In persons with normal arterial oxygen saturation the method of taking three samples, as advocated above, for the acetylene method should exclude the possibility of error   With care, moreover, the method will be found to be applicable to a large percentage of subjects with advanced cardiac disease and with mild congestive failure   In those subjects in whom the method is not applicable, the venous plateau method may be applied   Unfortunately, it is in these very cases that the pitfalls inherent in the latter method occur and great care and labor are required to avoid them   A further study of these cases is contemplated

SUMMARY

A critical study has been made of the possible sources of error of the Burwell-Robinson, venous plateau and acetylene methods for measuring the cardiac output which may invalidate the results obtained when applying them to patients with congestive heart failure   The methods to be used for detecting and avoiding these errors are indicated   Results as obtained by these different methods are given

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PHYSIOLOGICAL DISTURBANCES DURING EXPERIMENTAL DIPHTHERITIC INTOXICATION I BLOOD SUGAR, LACTIC ACID AND NON–PROTEIN AND AMINO–ACID NITROGEN [1]

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The therapy of diphtheria and control of the disease is largely based on outstanding achievements in the field of immunology, starting with von Behring's discovery of antitoxin, going on to Schick's method of measuring susceptibility to toxin and culminating in the development of means of producing active immunity by injection of a toxin antitoxin mixture Although the administration of antitoxin constitutes the chief mode of treating diphtheria, in a considerable number of severe cases this therapy alone is inadequate It was believed, therefore, that additional study of the metabolic disturbances of diphtheritic intoxication was indicated and might supply data which would suggest additional lines of treatment This and three subsequent papers (29, 30) will report studies in this field

Disturbances in carbohydrate metabolism during experimental and clinical diphtheritic intoxication have been the subject of intensive investigation In 1914, Rosenthal (1) demonstrated delayed sugar tolerance curves in rabbits following the injection of diphtheria toxin This was confirmed by other investigators (2, 3 4) With doses of toxin which lead to death in two to three days Rosenthal and others (4 5 6) frequently found markedly reduced fasting blood sugar values 12 to 24 hours before the death of the animals Lawrence and Buckley (7), injecting smaller doses of diphtheria toxin (causing death in from 6 to 8 days), noted the occasional occurrence of a pre lethal rise in the blood sugar Schwentker and Noel (6) demonstrated a similar tendency towards hyperglycemia in the less severely intoxicated animals They found a fairly close direct relationship between the period of survival and the elevation of the blood sugar Impairment of the normal blood sugar depressing action of insulin was demonstrated in rabbits poisoned with diphtheria toxin by Lawrence and Buckley (7) and by Netzley (8) Sweeney (4), on the other hand, could find no diminution in the response to insulin, under similar conditions

Investigations during the course of diphtheria in humans yielded results which are similar in many respects to those cited above Hector (9), in 1926, found delayed sugar tolerance curves in 5 of 6 patients These tests when repeated during convalescence were all normal Similar findings were reported

by Elkeles and Heimann (10), Prochajka (11), Schwentker and Noel (6), Benn, Hughes and Alstead (12) and Brems (13)   In occasional patients the fasting blood sugar values were found moderately elevated (9, 10, 13), in one patient reported by Elkeles and Heimann, as high as 400 mgm per cent   The latter authors also reported extremely low blood sugar values in some of their patients   The injection of insulin prior to the administration of glucose for the sugar tolerance tests was found by Elkeles and Heimann to have no appreciable effect upon the resulting curve   Schwentker and Noel, on the other hand, found that the injection of insulin definitely altered the response in the direction of the normal sugar tolerance curve

The occurrence of glycosuria during the clinical course of diphtheria has been noted   Hibbard and Morrissey (14) found reducing substances in the urine of 34 of 96 patients and in 8 of 9 fatal cases   The glycosuria in the nonfatal cases was transitory, disappearing during convalescence   Brems (13) also found spontaneous glycosuria in some of his patients   Elkeles and Heimann, on the other hand, failed to find urinary sugar in any of the cases, despite the presence of hyperglycemia

The bearing of the above investigations upon the nature of the disturbance in carbohydrate metabolism during diphtheritic intoxication has been the subject of much speculation   The suggestions that have been offered fall principally into two groups, namely   (1) a disturbance in the adrenals or related glands of internal secretion, (2) a depression or dysfunction of the insulin mechanism Rosenthal (1), Mikami (5) and Elkeles and Heimann (10) favored the former concept of adrenal damage, although Rosenthal felt that liver injury played an important but secondary rôle   Lawrence and Buckley (7), on the basis of histological evidence of injury to the thyroid and adrenals, implicated the latter glands as the responsible factors   Hector (9) considered that the basis for the disturbance lay in a derangement of the entire endocrine apparatus as well as moderate liver injury   The insulin mechanism was believed primarily responsible for the disturbances of carbohydrate metabolism by Sweeney (4), Sweeney and Lackey (3), Schwentker and Noel (6) and Benn, Hughes and Alstead (12)   The latter two groups of investigators utilized insulin and glucose as an adjunct to the antitoxin treatment of the disease with apparently good results

The literature on the nonprotein nitrogen constituents of the blood is less voluminous   In 1914, Karsner and Denis (15) found, following the injection of diphtheria toxin into cats, a slight increase of the nonprotein nitrogen during the first 2 days and then a marked rise up to 200 mgm per cent 1 or 2 days before death (4 to 6 days)   They attributed these results to the development of a nephritis, tubular in nature, during the early phase of the intoxication and vascular during the latter phases   Glesinger-Reischer and Glesinger (16) found the blood urea nitrogen greater than 30 mgm per cent in 16 of 21 patients with diphtheria and over 50 mgm per cent in seven   Following the administration of antitoxin, 20 to 50 per cent reductions in the urea values occurred in about half of the patients   Because of the essentially negative urinary findings they attributed the elevations in blood urea to the toxic destruction of body proteins   Prochajka (11) found that the nonprotein nitrogen of the blood was a most valuable prognostic sign in diphtheria patients   From a study of 86 cases he cited the following statistics   (1) when the nonprotein nitrogen was less than 40 mgm per cent, the mortality was 5 per cent, (2) when the nonprotein nitrogen was between 40 and 50 mgm per cent, the mortality was 32 per cent, (3) when the nonprotein nitrogen was over 80 mgm per cent, the mortality was 69 per cent

In experimental animals and patients with diphtheria Lereboullet, Gournay and Donato (17) found elevated blood urea but no disturbance in elimination of phenolsulphonephthalein nor marked histological changes in the kidneys They emphasized the fact that the degree of azotemia was directly proportional to the severity of the diphtheritic intoxication and believed that the elevation in blood urea was brought about by a suprarenal disturbance It is important to point out here that the phenolsulphonephthalein excretion test becomes difficult of interpretation in the presence of the severe liver damage that is usually found in diphtheritic intoxication Hanner and Whipple (18) showed that liver injury produced by chloroform and phosphorus poisoning was accompanied by a definite increase in the renal excretion of the dye A normal excretion during diphtheria therefore cannot be used as an indication of normal renal functions Derot (19) demonstrated, in rabbits injected with diphtheria toxin that the rise in blood nonprotein nitrogen was accompanied by a roughly parallel rise in blood creatinine

No reports of amino acid determinations in experimental diphtheritic poisoning or clinical diphtheria have been found in the literature

PROCEDURE AND METHODS

Rabbits weighing from 1400 to 2000 grams were employed in this investigation as the experimental animals The diet for approximately one week prior to the experimental period consisted of a liberal supply of greens and oats All food was removed from the cages 24 hours before the injection of the diphtheria toxin, and none was given during the balance of the experimental period Water was supplied in liberal quantity The diphtheria toxin used was found to contain 62 minimal lethal doses per cubic centimeter The toxin was diluted with sterile saline so that the calculated dose was contained in about 0 3 cc of solution This was injected intravenously

One series of animals received approximately three quarters, and another series two to three minimal lethal doses The smaller dose caused the death of the animals in from 4 to 7 days, the larger dose, in from 1 to 3 days The animals were bled by cardiac puncture every day or every other day, depending on the severity of the intoxication About 5 cubic centimeters of blood were drawn off and transferred to a bottle containing sufficient potassium oxalate to prevent clotting Each bottle also contained a small quantity of powdered sodium fluoride to inhibit glycolysis The Folin Wu method of precipitation was used to obtain a protein free filtrate

For the blood sugar determinations the Shaffer, Hartmann and Somogyi (20) method was employed Nonprotein nitrogen was determined colorimetrically by direct Nesslerization following digestion of the protein free filtrate with sulphuric acid and 30 per cent hydrogen peroxide Lactate was determined by the method of Avery and Hastings (21)

Amino acid nitrogen was determined by Folin's colorimetric method (22) It was found using the quantities prescribed by Folin, namely, 5 cc of the 1 10 filtrate and 1 cc of the 0 5 per cent solution of sodium β naphthoquinonesulfonate, that the largest concentrations of amino-acid nitrogen which could be determined with any degree of accuracy approximated from 6 to 10 mgm per 100 cc of blood Schmidt (23) made this same observation in a paper published in 1929 It was found most practicable in cases where there was doubt as to the approximate concentration of the amino acid nitrogen to set up a series of 3 tubes for each specimen of blood containing 1, 3 and 5 cc respectively of the filtrate and to add 2 cc of the reagent to each The tube in which the color

that developed was most closely matched by the standard, was used for the colorimetric comparison The error in the method is approximately 5 per cent in the lower values and 10 per cent when dealing with values in the vicinity of 40 to 60 mgm per cent

<div align="center">RESULTS</div>

The results are summarized in Tables I and II, and representative experiments are graphically presented in Charts 1 and 2 It is evident that the data confirm the findings of previous investigators as to the changes that occur in the blood nonprotein nitrogen and sugar during the experimental intoxications Briefly, the results can be described as follows
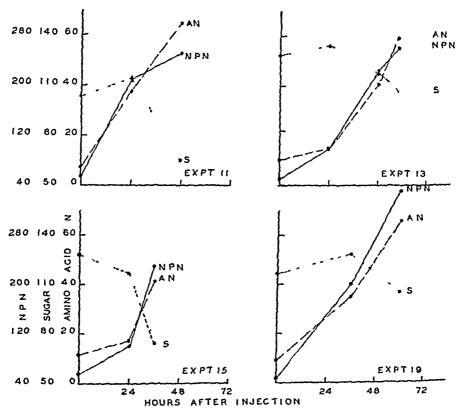
CHART 1 CHANGES OF BLOOD SUGAR, NONPROTEIN NITROGEN AND AMINO-ACID NITROGEN FOLLOWING THE INJECTION OF 3 MINIMAL LETHAL DOSES OF DIPHTHERIA TOXIN

After a relatively large dose of toxin, i e , one causing death in less than 3 days, there is a progressive increase in the nonprotein nitrogen which can be demonstrated as early as 24 hours after the injection, a definite tendency for the blood sugar to fall, in some cases, to relatively low levels, a rather abrupt rise in the amino-acid nitrogen (Chart 1) After a smaller dose of toxin, i e , one causing death in from 4 to 7 days, the changes are slower in appearing, but just as definite (Chart 2) These are a gradual, but progressive rise in the nonprotein nitrogen, a tendency for the blood

sugar either to change little or to rise to definite hyperglycemic levels, and a slight to moderate rise in the amino acid nitrogen early with in some cases a tendency for the amino acid nitrogen to decrease subsequently, or remain moderately elevated until the death of the animal
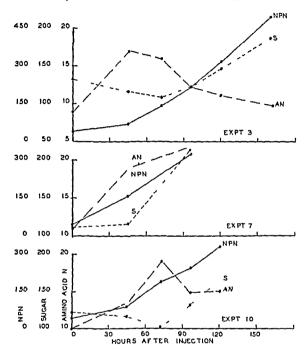

CHART 2 CHANGES OF BLOOD SUGAR, NONPROTEIN NITROGEN AND AMINO ACID NITROGEN FOLLOWING THE INJECTION OF 0 8 MINIMAL LETHAL DOSE OF DIPHTHERIA TOXIN

The nonprotein nitrogen rises at a regular rate with both doses of toxin Although the nonprotein nitrogen increases more rapidly in the animals receiving the larger dose of toxin it attains greater final concentration in the animals receiving the smaller dose of toxin Apparently the final height of the nonprotein nitrogen is dependent chiefly on the duration of the intoxication

The sugar and amino acid nitrogen values present an interesting relationship This can be more clearly observed in some of the individual experiments With the larger dose of toxin, as the blood sugar fell, the

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amino-acid nitrogen rose   For example, in Experiment 11 (see Chart 1), the blood sugar after 24 hours was 113 mgm per cent and the amino-acid nitrogen 35 mgm per cent   Coincident with the fall in sugar to 65 the amino-acid nitrogen rose to 55   Again, in Experiment 15, as the sugar fell from 117 to 73 the amino-acid nitrogen rose from 16 1 to 41 5   Similarly, in Experiment 13, the amino-acid nitrogen rose from 15 to 60 while the sugar fell from 135 to 108   In general it may be said that in the rabbits that die before the 3d day after having received relatively large doses of diphtheria toxin, the hypoglycemia is almost invariably accompanied by a marked amino-acidemia

In the experiments in which the rabbits received smaller doses of toxin and survived from 4 to 7 days, the situation was somewhat different   The changes can best be pointed out in the experiments in which the animals survived the longest, namely, Experiments 3 and 10 (Chart 2)   In these there is, at first, a very slight fall in the blood sugar values, coincident with a slight rise in the amino-acid nitrogen   Later, as the hyperglycemia develops, there is a tendency for the amino-acid to remain relatively stationary or to return to the pre-injection level   As has been pointed out, not all of the animals receiving the small dose of toxin develop hyperglycemia, nor do all show this relationship between the height of the blood sugar and the amino-acidemia as definitely as it has been described above   The significance of this will be discussed later

PATHOLOGY

A number of histological sections were made of the various organs of the animals used in the chemical studies   Although these sections do not demonstrate any facts not already recognized, they permit correlation of the anatomical changes with the various stages of diphtheritic intoxication manifested in the chemical disturbances   Briefly, the histological changes in the animals receiving the larger dose of toxin were as follows   (1) Necrosis of the liver parenchyma involving in some cases the major part of the liver cells   (2) A tubular and glomerular nephritis which in extreme cases led to necrosis of large areas of the kidneys   (3) Degeneration of heart muscle fibers   (4) Hemorrhages in various organs including the adrenals and intestines   The last finding was not constant, but occurred in the animals most severely affected   The findings in the rabbits receiving the smaller doses were similar but less acute and extensive   The myocardial fibers were occasionally surrounded by round cells and some repair was apparently taking place   The liver in these, also, showed necrosis, which in some cases was limited to the area surrounding the central vein, with dilatation of the liver capillaries which gave a picture indistinguishable from that of chronic passive congestion   The kidney lesions were definite but did not seem to be necessarily irreparable   Lesions of the adrenal parenchyma, except in the presence of hemorrhage, could not be recognized with certainty after either dose   All the animals showed a

certain degree of dehydration as evidenced by diminished skin turgor, a relative increase in the concentration of hemoglobin (30) and loss of weight of 10 to 20 per cent in typical cases   All these findings have been described in human cases   We would particularly call attention to the liver and kidney lesions which have been overlooked most frequently

DISCUSSION

The elevation of the nonprotein nitrogen can be most logically related to nephritis   This is confirmed also by the fact that the elevation of amino acid nitrogen does not parallel that of the nonprotein nitrogen   Undoubtedly dehydration aggravated the renal insufficiency   Control experiments demonstrated that starvation alone may lead to an elevation of the nonprotein nitrogen but this rarely exceeded 75 mgm per cent

Previous work indicates that liver injury is the chief cause of elevation of amino acid nitrogen   Marshall and Rowntree (24) found elevated blood amino acid nitrogen in the terminal stages of phosphorus and chloroform poisoning   Lewis and Izume (25) found a similar elevation in hydrazine poisoning in rabbits   Elevations of amino acid nitrogen are also reported in acute yellow atrophy (Rabinowitch (26)), and in experimental yellow fever (Wakeman and Morrell (27))   Apparently the margin of safety in the deaminization function of the liver is so great that only extreme destruction of the liver leads to increase in the amino acid nitrogen   Although an increase has been reported in rare cases of uremia, nephritis is not usually considered a cause of amino acidemia   The degree to which the amino-acid nitrogen increased in the rabbits given diphtheria toxin is greater than that reported in severe hepatic disease in humans, except in a few instances (Rabinowitch)   The data demonstrate marked disturbances of liver function corresponding to the extensive liver necrosis

We have been unable to find reports of blood amino acid nitrogen determinations in patients with diphtheria and have only had opportunity to make this determination ourselves in two cases of severe diphtheria   In neither was there an elevation of amino acid nitrogen although one patient developed definite myocardial degeneration and severe neuritis   Probably only a small number of the most severe cases will show an elevation of the amino acid nitrogen

With the recognition of the role of hepatic degeneration in severe diphtheria intoxication, the abnormality of carbohydrate metabolism receives an adequate explanation   With the greatest degrees of liver injury, the function of deaminization is diminished and presumably other related processes which serve to convert non carbohydrate material to glucose fail   The loss of stored glycogen and the breakdown of the processes of glyconeogenesis lead to hypoglycemia   In Paper II of this series (29a) failure of the liver injured by diphtheria toxin to store glycogen is demonstrated   These findings are in agreement with the work of Corkill (28)   This inability to store glycogen occurs in the animals receiving large and

small doses of toxin and can be demonstrated in animals at stages of the intoxication when no elevation of amino-acid can be demonstrated   With persistence of hepatic glyconeogenesis and impairment of hepatic glycogenesis, hyperglycemia develops   Since varying degrees of failure of these two hepatic functions may occur simultaneously, varying degrees of hypo- and hyperglycemia are found   As has been pointed out, hypoglycemia seems to be related to the rapidity of the increase of amino-acid nitrogen, and hyperglycemia to the period of survival when no increase in the amino-acid nitrogen occurs

The relation of failure of hepatic glyconeogenesis to accumulation of blood lactic acid is presumably close   An attempt was made to study this by daily blood lactic acid determinations   The data are presented in Tables I and II   One hesitates to draw any conclusions from this material because

TABLE I

Changes in blood sugar, lactic acid and nonprotein and amino acid nitrogen following the intravenous injection of 3 minimal lethal doses of diphtheria toxin

Experiment number	Period following injection of toxin	Nonprotein nitrogen	Sugar	Amino acid nitrogen	Lactic acid	Period of survival
	hours	mgm per cent	mgm per cent	mgm per cent	m Eq per liter	hours
		50	103	7 0	2 4	
	24	206	113	37 0	8 4	
11	48	250	65	65 0	12 7	50
		40	135	7 5	4 3	
12	24	170	77	37 0	14 8	28
		50	129	10 2	3 4	
	24	100	135	15 0	6 6	
	48	225	118	41 0	8 1	
13	54	265	108	60 0	9 0	59
		44	133	11 0	7 6	
14	24	90	72	27 5	9 7	34
		56	128	10 8	5 7	
	24	100	117	16 1	4 9	
15	36	220	73	41 5	5 8	40
		40	116	11 5	6 1	
16	24	92	63	32 0	7 2	27
		45	119	10 2	6 9	
17	24	100	82	27 8	10 3	32
		50	128	9 0	5 0	
18	24	133	101	29 5	7 2	36
		48	117	9 4	6 3	
	36	200	128	34 5	4 8	
19	60	350	106	66 0	12 0	63

TABLE II

Changes in blood sugar lactic acid and nonprotein and amino acid nitrogen following the intravenous injection of ¾ minimal lethal dose of diphtheria toxin

Experiment number	Period following injection of toxin	Nonprotein nitrogen	Sugar	Amino acid nitrogen	Lactic acid	Period of survival
	hours	*mgm per cent*	*mgm per cent*	*mgm per cent*	*m Eq per liter*	*hours*
		40	123	10 3	9 7	
	48	148	122	15 0	6 2	
	96	320	150	14 6	5 8	
1	120	410	158	11 4	6 8	128
		43	136	9 5	10 7	
	48	80	112	13 2	6 2	
	96	125	137	14 0	8 2	
	120	210	218	14 9	7 8	
	144	260	196	12 5	5 0	
2	172	380		12 8	5 0	176
		40	133	9 4	9 3	
	48	71	116	17 3	8 2	
	72	144	109	16 6	5 6	
	96	215	122	12 3	4 8	
	120	320	149	11 6	5 6	
3	168	500	187	10 0	7 7	184
		44	105	8 8	7 1	
	48	91	123	15 8	6 3	
	72	240	129	14 0	7 5	
4	96	450	109	16 9	6 5	97
		42	127	9 7	8 6	
	48	100	118	14 4	7 3	
5	72	380	160	16 0	5 0	90
		33	119	8 8	9 2	
	48	105	118	15 4	8 7	
6	96	350	122	21 0	12 7	104
		45	111	11 0	4 8	
	48	154	114	19 5	10 5	
7	96	330	215	22 0	9 2	104
		44	131	9 8	9 5	
	48	85	120	13 9	8 2	
8	72	230	132	34 0		82
		48	140	9 5	6 0	
	48	73	116	13 7	6 4	
	72	185	134	27 0		
9	96	300	237	32 0		98
		40	122	9 9	5 6	
	48	83	116	13 4	12 5	
	72	190	100	19 0		
	96	250	132	14 8		
10	120	340	165	15 2		128

of the wide variation in blood lactic acid levels in the normal animals The pre-injection levels were so frequently markedly elevated, due to struggling during the removal of blood samples from the hearts, that we regard lactic acid determinations in rabbits as unreliable indices of the state of blood lactic acid under resting conditions   In general, one may say that with the less severe intoxication there is less tendency for the lactic acid to reach the markedly elevated levels that are sometimes encountered in the severely intoxicated rabbits

A similar unreliability of the blood sugar values might be suspected However, essentially the same concentrations as those reported in this paper were found in blood taken from the ear when struggling was minimal This fact, together with uniformity of the trend of the results, leads us to consider the blood sugar values reliable

SUMMARY

1 The blood sugar, amino-acid nitrogen, nonprotein nitrogen, and lactic acid were studied in fasting rabbits which had intravenous injections of small and large doses of diphtheria toxin

2 During the severe intoxication, hypoglycemia, marked amino-acidemia and azotemia were found

3 A tendency was demonstrated for the hypoglycemia to occur while the amino-acid nitrogen was rapidly increasing

4 During the mild intoxication hyperglycemia could be demonstrated in most of the animals that survived for 5 or more days   Under these circumstances the amino-acid nitrogen was only slightly elevated   Nonprotein nitrogen became markedly elevated

5 It is pointed out that the azotemia is probably related to nephritis produced by the toxin and perhaps aggravated by dehydration   The amino-acidemia is apparently brought about by extensive liver necrosis The varying degrees of hypo- and hyperglycemia probably are related to varying degrees of failure of hepatic glyconeogenesis and glycogenesis

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PHYSIOLOGICAL DISTURBANCES DURING EXPERIMENTAL DIPHTHERITIC INTOXICATION   II HEPATIC GLYCO-GENESIS AND GLYCOGEN CONCENTRATION OF CARDIAC AND SKELETAL MUSCLE [1]

By HERMAN YANNET and DANIEL C. DARROW

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In the first paper of this series (12) it was pointed out that during diphtheritic intoxication in rabbits there was evidence of liver disturbance, manifested by a marked rise in the amino acid nitrogen which in the more severe intoxications was accompanied by a hypoglycemia   When smaller doses of toxin were injected the amino acid nitrogen changed but little, while the blood sugar remained relatively normal or rose to hyperglycemic levels   Accompanying these changes the nonprotein nitrogen rose independently of the amino acid nitrogen, a finding which seemed to be associated with the nephritis which could be demonstrated histologically

In order to associate, if possible, these findings of hypoglycemia, hyperglycemia and normal sugar values with liver injury, the assumption was made that the two chief functions of the liver concerned with carbohydrate metabolism may be injured separately or in varying degrees   It was felt that the failure of deamination as indicated by the elevated amino-acid nitrogen concentrations could be interpreted as a type of injury that might be associated with the failure of hepatic glyconeogenesis leading to hypoglycemia   However if the formation of glucose from non carbohydrate material is only slightly impaired, a failure of hepatic glycogenesis would result in hyperglycemia

The following experiments were designed to study the ability of rabbits suffering from diphtheritic intoxication to store glycogen in the various tissues   The effect of intravenous injections of glucose with and without insulin, on the liver and heart glycogen concentrations, is reported

PROCEDURE AND METHODS

Rabbits weighing about 1500 grams were injected intravenously with 3/4 to 1 minimal lethal dose of diphtheritic toxin   This dose caused death in from 3 to 7 days   The rabbits were on a stock diet consisting of oats and greens until 24 hours before the injection of the toxin when the food was removed

from the cages   Starvation was maintained throughout the balance of the experiment, water was offered freely   The level of the blood nonprotein nitrogen was used as a fairly reliable index of the severity of the intoxication, this was determined daily and when it reached 100 to 150 mgm per cent or higher the operative phase of the experiment was started   (Previous experience indicated that diphtheritic rabbits with a blood nonprotein nitrogen level of this magnitude die within 24 to 48 hours)   As an anesthetic, one-half cubic centimeter of a 10 per cent freshly prepared amytal solution per kilogram of body weight was injected intraperitoneally   Following the development of adequate anesthesia the liver was exposed by a mid-line upper abdominal incision   A section of liver weighing approximately 3 to 5 grams was removed, bleeding being effectively controlled by a ligature, and was immediately cut into small pieces and dropped into a weighed tube containing 60 per cent potassium hydroxide for glycogen determination   In one series of animals 5 cc of a 50 per cent glucose solution was then injected intravenously immediately, and again after an interval of 20 minutes   In another series the same technique was employed except that 2 1/2 units of insulin were injected along with the glucose solution   One hour following the last administration of glucose another section of liver was obtained for glycogen determination   (Preliminary experiments had shown that the increase of liver glycogen was approximately the same after one as it was after two hours)   The hearts were removed at the same time as the second liver section   The controls were subjected to precisely the same procedure, including the starvation regime, for a similar period of time

Data referable to the glycogen content of liver, heart and skeletal muscle were obtained from another series of animals on a similar fasting regime   The tissues were removed within 30 seconds after the animal was stunned by a blow at the base of the skull   At the time of these determinations the blood nonprotein nitrogen of the diphtheritic animals was about 150 to 200 mgm per cent   The same dose of toxin was used in this series as in the one previously described

The glycogen method employed was fashioned after Pfluger's original procedure but was sufficiently modified to warrant description   About 5 grams of tissue were cut into small sections as soon as possible after removal from the animal and collected in a weighed centrifuge tube of 100 cc capacity containing 10 cc of 60 per cent potassium hydroxide solution, which was then reweighed   Digestion was allowed to take place in a steam bath for a period of three hours, or until a relatively clear liquid resulted   Approximately 75 to 80 cc of 95 per cent alcohol were then added and the tube allowed to remain in a refrigerator overnight to insure complete glycogen precipitation   The tube was then centrifuged at moderate speed for 10 minutes, the supernatant fluid siphoned off and the glycogen precipitate washed with 25 cc of 95 per cent alcohol   Following another centrifugation for 10 minutes the supernatant fluid was again removed   One drop of phenolphthalein and 25 cc of water were added and the resulting solution neutralized by the careful addition of 25 per cent hydrochloric acid   (This usually required 4 to 5 drops)   Two and one-half cc of the acid were then added, producing a final acidity approximately equivalent to a 2 1/2 per cent hydrochloric acid solution   The glycogen was then hydrolyzed by heating in a steam bath for three hours   The resulting solution was neutralized and made up to a suitable volume and the glucose content determined in aliquots by Somogyi's modification of the Shaffer-Hartmann sugar method   The glycogen content is expressed as milligrams of glucose per gram of tissue   The method has an error in duplicates of about 8 per cent

RESULTS

In Chart 1 are graphically tabulated the results of the determinations of the glycogen content of liver, muscle and heart, in both diphtheritic and normal animals   There are two striking observations to be made   first, that there is essentially no difference in the glycogen content of the heart and skeletal muscles of the diphtheritic as compared to the normal animals   second, that there is a definite diminution in the glycogen content of diph theritic livers as compared to normals
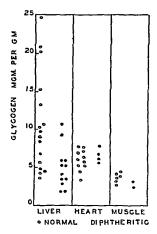

CHART 1   THE GLYCOGEN CONCENTRATION OF LIVER HEART AND MUSCLE IN NORMAL AND DIPHTHERITIC RABBITS

The glycogen content of normal and diphtheritic livers before and after the injection of glucose and glucose plus insulin is graphically represented in Chart 2   The glycogen concentration of the liver before and after the glucose and glucose plus insulin injections is represented by a circle and arrow end respectively   The length of the intervening line represents the actual increase in glycogen concentration in milligrams per gram of tissue In the normal animals the injection of glucose brought about an increase in the glycogen content of the liver which varied from 6 to 29 mgm per gram of tissue, in other words an increase of from approximately 0 5 to 2 per cent in glycogen concentration   The addition of insulin, it will be seen, had no appreciable effect in normal animals a finding which is simi lar to that described by other investigators

In the diphtheritic animals on the other hand, following the injection of glucose the increase in liver glycogen varied from less than 1 mgm to

In Chart 3 the results of the glycogen determinations on the hearts of the normal and diphtheritic animals, following the intravenous injection of glucose, with and without insulin, are represented   These have been compared to the values found in the untreated animals   There is no significant difference in the cardiac glycogen of normal and diphtheritic animals following injections of glucose   However, the cardiac glycogen of diphtheritic animals following the injection of glucose plus insulin may be significantly greater than in the other groups

<center>DISCUSSION</center>

Early in this investigation it was appreciated that diphtheritic rabbits refuse all food relatively soon after receiving an injection of toxin and should, therefore, be regarded as fasting animals   Since one of the striking effects of starvation is a depletion of the glycogen stores, it was apparent that in a comparative study of glycogen content of various tissues in normal and intoxicated animals, one would have to consider not only the effect of the diphtheria toxin but also of starvation   It was felt that the most practical procedure for minimizing this difficulty was to maintain both groups of animals on a similar fasting regime   We thus felt that any differences encountered in the glycogen concentrations of the examined tissues could with greater reliability, be attributed to the effect of the diphtheria toxin alone

Although diphtheria both clinically and experimentally, is a generalized disease process and not limited to any one particular organ it is nevertheless true that the myocardial involvement is an outstanding and relatively frequent manifestation of a severe intoxication   Warthin (1) in his review of the anatomical changes in the heart in diphtheria suggested the possibility that some of the encountered lesions may well be related to a nutritional disturbance   Schwentker and Noel (2), who, on the basis of their experimental and clinical investigations and a review of the literature, stressed the theory of insulin dysfunction as the cause of the carbohydrate disturbance, believed that failure of glycogenesis played an important role in the myocardial insufficiency   It was therefore with interest that we noted no essential difference in the glycogen concentration of the hearts of diphtheritic and normal animals   As far as we can determine from a review of the available literature there have been no determinations of heart glycogen in diphtheritic animals

The few determinations of heart glycogen before and after injection of glucose cannot be interpreted with certainty   There is no evidence that the amount of glucose injected increased the heart glycogen in either the diphtheritic or normal animals   The group of diphtheritic animals subjected to intravenous injection of glucose plus insulin have slightly higher heart glycogens than the controls   The difference in the averages is just three times the probable error of the differences   However, in a small

number of determinations such a difference cannot be regarded as more than suggestive

An occasional reference to muscle glycogen determinations in experimental diphtheritic intoxication is found in the literature   Schwentker and Noel found the muscles of three animals completely devoid of glycogen and in a fourth, 0 16 per cent   Rosenthal (3) in three rabbits found the muscle glycogen to be 0 165, 0 245 and 0 305 per cent respectively   We have found in six animals the glycogen concentration to vary from 0 23 to 0 43 per cent, but we have also found that in six normal animals under a similar starvation régime the muscle glycogen was approximately of similar magnitude, varying from 0 26 to 0 42 per cent   In other words, as in the case of cardiac glycogen, no essential difference could be demonstrated between the muscle glycogen of normal and diphtheritic animals

An entirely different picture is presented when one examines the values for the glycogen concentration of the livers in the diphtheritic animals In this case there is a definite decrease in the intoxicated rabbits, the glycogen content varying from 0 12 to 0 63 per cent, and averaging about 0 35 per cent, while in the normal but fasted rabbits the concentration in the liver varies from 0 4 to 2 0, averaging about 1 0 per cent   Whether the liver shares to a greater extent in the general systemic intoxication than does either the heart or muscle, as these data would seem to imply, is not absolutely clear   Histologically the liver exhibits more injury than the cardiac or skeletal muscle   However, it is probable that a large part of the liver glycogen serves primarily as storage material, and that it is the diminution in this fraction which accounts for the marked difference in the glycogen concentration encountered   On the other hand in the heart and to a lesser extent in muscle, most of the glycogen content is intimately related to the immediate metabolic needs of the tissues in question, and is, therefore, less likely to be involved in an injury of relatively similar degree than is the glycogen of the liver

The data obtained in the study of hepatic glycogenesis show a marked disturbance in the ability of the diphtheritic liver either to store or to form glycogen or both   Furthermore, the injection of insulin does not correct this deficiency   Numerous investigators have demonstrated a marked decrease in carbohydrate tolerance as evidenced by diabetic-like sugar tolerance curves   Moreover, Lawrence and Buckley (4), Netzley (5) and Sweeney (6) in animals, and Elkeles and Heimann (7) and Schwentker and Noel (2) in human cases of diphtheria, have found a decided impairment in the normal depressing action of insulin on the sugar tolerance curves   As was previously pointed out, this has been interpreted by some to signify insulin dysfunction or actual insufficiency   It is apparent that an inability of the liver to remove glucose for storage as glycogen would adequately explain a delayed or elevated sugar tolerance curve and apparent refractoriness to insulin   Obviously, such a failure of hepatic glycogenesis

does not necessarily signify any inability to burn carbohydrate   In the third paper of this series we demonstrate by means of respiratory quotients determined during the course of the intoxication that diphtheritic rabbits can burn carbohydrate

The occurrence of severe liver injury during diphtheritic intoxication is by no means unrecognized   Pathologically this was noted many years ago by Babes (8), Welch and Flexner (9) Fahr (10), and others   Histologically, the lesion consists essentially of varying degrees of necrosis in the region of the central vein   From our own experience in examining numerous sections of liver obtained from diphtheritic animals, this has been the most constant and striking pathological finding   In many cases the destruction was so extensive that very little normal hepatic tissue could be seen

It may be pertinent, in this connection, to point out the striking similarity which exists, as far as evidence of disturbance in carbohydrate metabolism is concerned between diphtheritic intoxication and other conditions in which liver injury is the principal pathological finding, namely, phosphorus, chloroform and hydrazine poisoning and yellow fever   In these conditions there have been demonstrated hypoglycemia, diabetic-like sugar tolerance curves marked reduction in liver glycogen and increases in blood amino-acid nitrogen (11)

SUMMARY

1 The liver glycogen of fasted rabbits injected intravenously with diphtheria toxin is definitely diminished in comparison with that of controls subjected to similar periods of fasting   Under these conditions no significant difference was noted in the concentration of glycogen in heart and muscle of diphtheritic and control rabbits

2 A marked diminution in hepatic glycogenesis following the intravenous injection of glucose was noted in diphtheritic rabbits   The injection of insulin along with the glucose did not alter hepatic glycogenesis

3 The concentrations of heart glycogen of normal and diphtheritic rabbits following the injection of glucose with and without insulin, showed no significant differences

4 It is pointed out that the destruction of liver parenchyma demonstrated anatomically in diphtheritic intoxication is accompanied by a failure of hepatic glycogenesis which is responsible for some of the disturbances in carbohydrate metabolism described in this condition

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The data obtained in the study of hepatic glycogenesis show a marked disturbance in the ability of the diphtheritic liver either to store or to form glycogen or both   Furthermore, the injection of insulin does not correct this deficiency   Numerous investigators have demonstrated a marked decrease in carbohydrate tolerance as evidenced by diabetic-like sugar tolerance curves   Moreover, Lawrence and Buckley (4), Netzley (5) and Sweeney (6) in animals, and Elkeles and Heimann (7) and Schwentker and Noel (2) in human cases of diphtheria, have found a decided impairment in the normal depressing action of insulin on the sugar tolerance curves   As was previously pointed out, this has been interpreted by some to signify insulin dysfunction or actual insufficiency   It is apparent that an inability of the liver to remove glucose for storage as glycogen would adequately explain a delayed or elevated sugar tolerance curve and apparent refractoriness to insulin   Obviously, such a failure of hepatic glycogenesis

PHYSIOLOGICAL DISTURBANCES DURING EXPERIMENTAL DIPHTHERITIC INTOXICATION III RESPIRATORY QUOTIENTS AND METABOLIC RATE [1]

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Previous investigators have demonstrated disturbances of carbohydrate metabolism during both clinical and experimental diphtheritic intoxication These disturbances are made manifest by delayed sugar tolerance curves, high blood sugar values, and, occasionally, glycosuria These observations have led to the assumption on the part of many that, during the course of diphtheria, there exists a relative insulin deficiency or dysfunction, a detailed review of this phase of the literature is given in Paper I of this series (1) In the two preceding papers of this series (1, 7) we have demonstrated by histological and chemical studies, a widespread destruction of liver parenchyma with derangement of liver function following the intravenous injection of diphtheria toxin in rabbits Physiologically, this manifested itself by varying degrees of disturbance in hepatic glyconeogenesis and hepatic glycogenesis The belief was expressed that the above mentioned disturbances in carbohydrate metabolism could be related to the hepatic injury In the following communication are reported the results of a study of the respiratory quotients in rabbits, aimed to determine what effect, if any, diphtheritic intoxication has upon their ability to burn carbohydrates

METHOD

The apparatus used for the determination of respiratory quotients was described by Marine (2) in 1922 and is based on the Haldane open-circuit system It was adequately tested before the experiments were begun for proper absorption of carbon dioxide and water and for air tight connections

Rabbits in good condition, and weighing from 900 to 1400 grams were employed for the investigation Both control and diphtheritic animals were deprived of food 24 hours before the onset of the experiment Starvation was maintained throughout the balance of the experiment only water being offered The diphtheria toxin used was of a strength such that 0 016 cc was equivalent to 1 minimal lethal dose of toxin Three quarters to 1 minimal lethal dose was injected intravenously It was previously found that this dose would bring about the death of the fasted animals in from three to seven days The animals

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TABLE II

Average respiratory quotients obtained in normal and diphtheritic rabbits after different periods of fasting

Hours of starvation	Diphtheritic		Normal	
	Average respiratory quotient	Number of observations	Average respiratory quotient	Number of observations
24	81	4	84	3
48	80	4	82	4
72	78	3	76	4
96	80	2	76	5
120+	78	2	73	1

to make the values express surface area in the ordinary units The omission of this procedure still allows comparison of the various animals in terms analogous to those which would be obtained with a more exact formula for surface area

The respiratory quotients determined during the course of the intoxication do not suggest any gross interference with the burning of carbo hydrates It can be seen that after the development of a fasting status the respiratory quotients tend to remain with only slight exceptions at a fairly constant level, which averages between 78 and 80, a level well above that to be expected had there been any severe degree of insulin dys function

In Table II the respiratory quotients obtained on the diphtheritic animals after similar periods of fasting have been averaged and compared with those obtained on the normal controls The lack of any striking difference between the two sets of values is apparent

It might be mentioned in passing, that no great change was noted in the total metabolic rate as the intoxication progressed In other words diphtheria toxin when injected into rabbits does not exhibit any calorigenic activity

DISCUSSION

Essentially the same approach for the investigation of the disturbance in carbohydrate metabolism during diphtheria has been utilized previously by Josephs (3), Hector (4) and Arloing and Laulanie (5)

Josephs determined the respiratory quotients in dogs by collection of the expired air over periods of from three to five minutes, followed by analysis of a sample in a Haldane gas apparatus The results of four experiments were described From the data presented it is evident that there was no difficulty in the oxidation of carbohydrates the nonprotein respiratory quotients remaining about 0 80 throughout the course of the investigation The dogs were subjected to only a mild degree of intoxication as evidenced by the relatively low blood nonprotein nitrogen levels and the prolonged periods of survival

Arloing and Laulanie in 1895 studied the respiratory exchange by means of closed calorimetry in rabbits following the injection of large doses of toxin subcutaneously (death occurred in from 6 to 36 hours) Because of the overwhelming nature of the intoxication it is difficult to interpret the results   Furthermore there was no suitable dietary control previous to the experiment   Respiratory quotients ranging from 0 90 to 0 70 were obtained   A tendency for a slight rise to occur during the first six to eight hours following the injection of toxin was noted

Hector in 1926 studied the respiratory quotients in six cases of diphtheria of varying degrees of severity, using a Fridericia apparatus   He determined the respiratory quotient at frequent intervals following the administration of glucose by mouth   From his results he concluded that there was no evidence of any inability to oxidize carbohydrates during the course of diphtheria

J A Johnston (6) has recently acquainted us with data similar to Hector's, on a diphtheria patient with severe myocardial involvement   The observations, made on the 22d day of the disease, showed that the respiratory quotients following the intravenous administration of 20 grams of glucose rose from the pre-injection level of 0 84 to 0 90 at one hour and fell to 0 85 and 0 77 at the second and third hours respectively   Comparing this with similar studies on normal children, Johnston interprets these results to signify an adequate response to the injected glucose   These observations were made too late in the disease to be strictly comparable to our experimental results   Similar studies, both early and late in the disease, would be valuable, especially if accompanied by observations of the blood nonprotein nitrogen, which seems to be the best objective measure of the severity of the intoxication

In Paper IV of this series (8), the results of the investigation of the serum electrolytes during diphtheritic intoxication in rabbits will be presented   These studies demonstrate the frequent occurrence of a decrease in serum bicarbonate, chloride and sodium   It is apparent that the development of acidosis while in the respiratory chamber would increase the respiratory quotient   However, it is not likely that any marked change occurred during the relatively short period of observation   Furthermore, it is unlikely that low respiratory quotients were so masked by developing acidosis as to bring about the substantial agreement in the respiratory quotients found in our experiments

The criticism which may be made of the work reviewed above, as well as of our experiments, is that by this type of investigation one can adequately demonstrate only severe grades of interference with carbohydrate oxidation   The failure to find any evidence of this disability would, therefore, not preclude minor degrees of insulin insufficiency or dysfunction   We believe this to be a just criticism and have duly considered it in framing our conclusions

SUMMARY AND CONCLUSION

1 Study of the respiratory quotients of rabbits during the course of diphtheritic intoxication produced by the intravenous injection of diphtheria toxin reveals no evidence of any gross disturbance in the oxidation of carbohydrates

2 It was not possible to demonstrate any significant changes in the total metabolic rate during the course of the intoxication

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STUDIES OF TOTAL PULMONARY CAPACITY AND ITS SUB-DIVISIONS I NORMAL ABSOLUTE AND RELATIVE VALUES

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In the investigation of the symptom, dyspnea by physiological methods studies of the pulmonary mechanisms have lagged behind those of other mechanisms involved In most clinics the measurement of the vital capacity is frequently made, but rarely in conjunction with measurements of total pulmonary air and its subdivisions No complete understanding of the problem can be attained without a complete study of the functions of the lungs in addition to those of the blood and the circulatory organs The studies to be reported here were commenced as a preliminary to an investigation of the functional disability caused by various types of fibrosis of the lungs, particularly the pneumonoconioses At the outset it was discovered that data regarding lung capacity were available in only a small number of normal individuals, so small that no standards of normality could be established for comparison with measurements to be made in pathological conditions

We will not attempt to make a historical review of the methods used in the determination of the lung volume and its subdivisions A complete presentation of the subject, from this point of view, has been published very recently by Christie (4) We may mention that probably three factors have largely been responsible for hesitancy in accepting this procedure as a useful tool in clinical medicine unreliability of the methods used, difficulty of using complicated methods in dealing with very sick patients, and finally lack of normal standards for comparative purposes The first two drawbacks have been solved by the methods described by Van Slyke and Binger (12) and lately by Christie (4) The present study was undertaken in an attempt to furnish some information regarding the normal values of the pulmonary air and its subdivisions, to appreciate the character of its variations to establish a method which will permit the prediction of normal values in a given case and finally to apply the information gained from the normal observations to the study of cases of

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chronic pulmonary pathology   In this and subsequent papers we will present our results, together with a consideration of the related literature

NOMENCLATURE

A serious drawback to the proper comparison of different series of observations has been the use of different terms, and even different meanings for the same term   It is therefore essential to adopt a single nomenclature in order to have a clear understanding of the subject   We have adopted for our studies the classification proposed by Christie (4) with the exception of the replacement of the term " functional residual air " by mid capacity   The classification may be briefly summarized as follows

Residual air is the amount of air remaining in the lungs after fullest possible expiration

Mid capacity is the amount of air remaining in the lungs after a normal expiration   The term " functional residual air " introduced by Lundsgaard and Schierbeck (6), and used by Binger and Brow (3), is synonymous with this term   It appears to be more convenient, however, to use the term mid capacity as being more descriptive and more commonly used   The term " functional residual air " may be easily confused with residual air, or it may suggest that it is a subdivision of the latter   Mid capacity represents the sum of the residual and reserve airs

Vital capacity is the amount of air expired in the fullest possible expiration following the deepest possible inspiration   Vital capacity is the sum of the complementary and reserve volumes

Total capacity of the lungs is the sum of the residual air and the vital capacity

Complementary air is the volume of air inspired from the position of mid capacity to that of the maximum possible inflation   It includes the tidal air

Reserve air is the amount of air expired from the mid capacity position to the maximum possible deflation

The character of this classification may be appreciated more readily in the diagram of Figure 1   Other classifications differ mainly in the definition of the mid capacity   Panum (9), in 1868, regarded this as the volume of air in the lungs at a point mid-way between normal expiration and inspiration   Most investigators have adopted his definition   It seems better, however, to define mid capacity as proposed by Siebeck (11), who took into consideration the commonly accepted finding that the most constant level in any graphic respiratory tracing is found at the end of a normal expiration   (Christie calls this level the " resting respiratory level ")
The level at which the mid capacity is placed in the different classifications changes the values given to the complementary and reserve volumes   It will be noted that in the nomenclature adopted for this study the complementary air is the volume of air from the level of normal expiration up to

the limits of complete inspiration (consequently it includes the *tidal air* which is the amount of air moving in and out during quiet breathing) The reserve air is the volume of air between the level of normal expiration and the limits of complete expiration
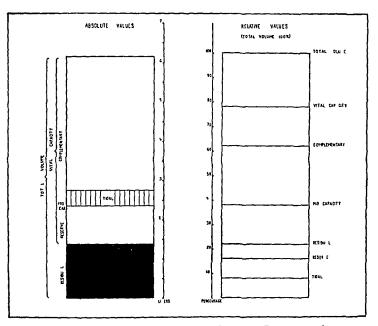

Fig 1 Mean Absolute and Relative Values of Pulmonary Air in 50 Normal Males

METHODS

We have used Christie's method (4) of oxygen dilution without forced breathing The details of this method have been fully described in this author's original description, so we will omit a detailed account The spirometer employed was of the type described by Van Slyke and Binger (12) of nine liters capacity, provided with a solid core to decrease the dead space with a graduated millimeter scale and a recording pen (each millimeter on the scale being equivalent to a volume of 20 cc) A five way valve permitted easy and quick communication of the patient with the desired connection The dead space of the spirometer, including rubber tubing soda lime container etc was calculated according to the instructions given by Christie, and found to be 2600 cc Care was taken to keep the same level of water in the spirometer by means of a small syphon mounted on a graduated scale The oxygen used

was analyzed to discover inert gases acting as impurities   Five liters were admitted to the spirometer for each determination   The respiratory tracings were recorded on paper with millimeter rulings, mounted on a kymograph drum   From the tracings (the patient being under basal conditions) the basal metabolic rate may be calculated, if desired   All our results have been corrected to the volume corresponding to a temperature of 37° C, and complete saturation with water vapor   The main defect of this method, as pointed out by Christie, is the difficulty in making an accurate measurement of the oxygen consumption   Although in most cases a satisfactory base line is secured, there are cases in which breathing is somewhat irregular, and in consequence only an approximate determination of the oxygen consumption may be obtained   The error under these conditions may be considerable   In such cases we have used the following procedure for the determination of the *residual air*   A second spirometer containing about three or four liters of atmospheric air, and provided with a recording pen, is connected by means of a wide and short rubber tube to one of the outlets of the five way valve   The kymograph is placed so that a graphic tracing may be obtained from the excursions of this spirometer   The subject breathes through the five-way valve to the outside air for one or two minutes   Then he is rapidly connected with this spirometer   After three or four breaths, at the beginning of a normal expiration, he is suddenly asked to continue to expire as completely as possible to the level of the maximum deflation, and to hold his breath at this level for just the time (about a second) necessary to turn the valve connection to the regular mixing spirometer containing the measured amount of oxygen   Quiet breathing is continued for seven or eight minutes and respirations are recorded on the kymograph to which the recording pen of this spirometer is adjusted as soon as possible after the connection is made   At the end of this period the subject is again suddenly asked to continue as full an expiration as possible and to hold the breath at this level while the connection to the spirometer is quickly closed off   The oxygen consumption is calculated directly from the graduated scale of the spirometer (from the difference between the level after the known amount of oxygen was added and the level at the end of the determination)   The respiratory tracing serves chiefly to detect any possible leakage or gross irregularity   It also gives a graphic record of the reserve air when the subject was asked to expire to the residual level at the beginning and end of the determination   The degree of the subject's cooperation can thus be readily determined by comparing these tracings, and proper corrections may be introduced, obviating, at least in part, the main error in the direct determination of the residual air   A third determination of the reserve air a few minutes later was sometimes used as a further check   A preliminary explanation usually suffices to obtain the desired cooperation   The rest of the calculation is made exactly as described by Christie, but of course the result obtained represents the residual air   This added to the reserve air will give the mid capacity

Our gas analyses have been made in the Van Slyke manometric apparatus (10)   This procedure is less time consuming than the use of the Haldane gas analysis apparatus   It has been found to give close agreement in duplicate analyses   All the determinations have been made with subjects in the recumbent position in bed, with two flat pillows for a head rest   In each case two determinations were made   one of mid capacity, following strictly Christie's method, and the other by the alternate method of determining the residual air according to the procedure just described   In most cases, when the base line for measurement of the oxygen consumption was satisfactory, a close agree-

ment was found between the volume of the residual air obtained directly and the one calculated by subtracting the reserve volume from that of the mid capacity   The vital capacity, reserve and complementary airs were determined immediately afterward in the same spirometer without change in the subject's position   After connection was again established with the spirometer, the subject was asked to breathe normally for one or two minutes and then while watching the graphic registration of his breathing he was asked to continue an expiration down to the maximum point of deflation to measure the reserve air   After quiet breathing was again resumed for a short time he was asked to continue an inspiration up to the maximum level of inflation thus measuring the complementary air   Finally after a short interval, we obtained a graphic tracing of the fullest possible inspiration followed by the maximum possible expiration (vital capacity)   In normal people this vital capacity ought to be equal to the sum of the reserve and complementary volumes determined separately   Not infrequently small differences are found, possibly due to lack of cooperation

MATERIAL

Determination of the total pulmonary air and its subdivisions has been made in 50 normal males, varying in age from 18 to 30 years and with a mean age of 23 years   The physical characteristics of these subjects are summarized in Table 1, from which it can readily be appreciated that no

TABLE 1
Physical characteristics of the subjects examined

	Mean	Standard deviation	Coefficient of variation	Variations
			per cent	
Age *years*	22 9 ±0 31*	3 3 ±0 22*	14 4	18–30
Body height *cm*	176 2 ±0 49	5 1 ±0 35	2 9	157 6 –186 5
Body weight, *kgm*	72 5 ±1 07	11 2 ±0 76	15 5	52 8 –104 0
Body surface area *cm²*	187 8 ±1 19	12 5 ±0 84	6 6	1 52– 2 19
Chest circumference *cm*	85 4 ±0 60	6 3 ±0 43	7 3	74–110
Chest volume *liters*	10 00±0 19	2 03±0 14	20 3	7 00– 18 33
Chest index $\frac{Depth}{Width} \times 100$	68 5 ±0 55	5 8 ±0 39	8 4	58 3 – 84 4
Chest index $\frac{Height}{Width} \times 100$	70 8 ±0 71	7 3 ±0 49	10 3	50–84
Costal angle °	71 8 ±1 18	12 4 ±0 84	17 2	50–104

* Probable error

selective criteria were used in regard to the bodily appearance   The chest size, as judged from external measurements, also varies within wide limits as well as its shape (as indicated by the variations in the indices of Depth/Width and Height/Width and in the costal angle)   This group of normal males although not very large in number, is accordingly representative and may be considered as a fair sample of this age period   The

subjects were students of the School of Medicine and the College for Men of the University of Rochester In all cases a brief history of previous diseases and athletic activities was recorded, and the chest examined clinically and radiologically The vast majority of the subjects were in nonfasting condition at the time of the determination, but all of them had a preliminary resting period of at least twenty minutes The total number of observations was fifty-two We have excluded from this report only two cases in one the value for residual air was found to be a negative quantity for some unknown reason, and in the other case satisfactory cooperation could not be obtained

TABLE 2
Values of pulmonary capacity from the literature *
Absolute values

Pulmonary capacity	Mean	Standard deviation	Coefficient of variation	Variations
	liters	*liters*	*per cent*	*liters*
Total capacity	5 98	1 12	18 7	3 25–8 22
Vital capacity	4 46	0 87	19 5	2 31–5 95
Complementary air	2 60	0 63	24 2	1 61–3 95
Reserve air	1 98	0 51	25 7	0 70–2 92
Mid capacity †	3 70	0 66	17 8	2 23–5 08
Residual air	1 50	0 33	22 0	0 87–2 48

Relative values (Total capacity = 100 per cent)				
	per cent	*per cent*	*per cent*	*per cent*
Vital capacity	75 1	4 2	5 6	60–84
Complementary air	40 6	4 9	12 0	26–48
Reserve air	30 0	6 2	20 6	15–40
Mid capacity	60 7	4 0	6 6	52–69
Residual air	25 3	4 3	17 0	16–40

Relative values (Vital capacity = 100 per cent)				
	per cent	*per cent*	*per cent*	*per cent*
Complementary air	56 7	5 9	10 4	44–69
Reserve air	44 0	5 9	13 4	31–56
Mid capacity	81 2	8 7	10 7	63–97
Residual air	34 2	11 0	32 1	19–66

* Summary of 46 determinations made on male subjects collected from Lundsgaard and Van Slyke (7), Lundsgaard and Schierbeck (6), Binger (2) and Anthony (1)
† Volume of air in the lungs mid-way between a normal expiration and inspiration

Normal values given in the literature

Records of normal values for the total capacity and its subdivisions are few, and it is difficult to evaluate them since the observations were made

with different techniques and under varying conditions Information about the age and physical characteristics of the people examined was usually meager Frequently values obtained in males and females have been combined so that it is difficult to separate the results according to sex We have summarized in Table 2 all the determinations made on normal *males* since Lundsgaard and Van Slyke (7) reported 11 observations in 1918 No attempt has been made to include earlier data, since the methods used are open to question We have included 11 cases of Lundsgaard and Van Slyke 19 cases of Lundsgaard and Schierbeck (6) the largest series in the literature 7 cases reported by Binger (2) and finally 9 cases from Anthony s report (1), making a total of 46 cases The determinations have been made either standing or sitting up, and the classification used by the authors quoted places the mid capacity halfway between normal expiration and inspiration In 11 cases, of the 19 reported by Lundsgaard and Schierbeck, we have found that the reported value of mid capacity is equal to the sum of the residual air and half the vital capacity, evidence that different criteria have been used in the calculation of that subdivision From the series of Anthony we have included only the values reported for residual air Table 2 has been arranged to present the summary of these data in absolute values for the different components of the total capacity, as well as their proportional relationship to the total volume and vital capacity Comparison of results obtained from the literature with our findings is made elsewhere in this paper

RESULTS

In Table 3 are summarized the determinations of the total pulmonary air and its subdivisions, indicating the mean values the deviations from the mean and the extreme variations The observed values are given, and relative values are expressed as percentages of the total volume and vital capacity

Absolute values observed The mean value of the *total capacity* in this series is 6 13 ± 0 08 liters, a figure very close to that of 5 98 liters, which is the corresponding mean of all the observations collected from the literature There are wide variations from this mean The standard deviation is 0 82 liter, with a coefficient of variation of 13 3 per cent, indicating that in our series there is a total range of variability of about 27 per cent from the mean value Similar, and even higher, variability becomes evident from the study of the reported observations in the literature

When we come to the *vital capacity* we also find, as others have found, a wide variation in the absolute values With a mean value of 4 78 ± 0 06 liters it varies between 3 40 and 5 85 liters, with a total variation of about 25 per cent from the mean The fact that all our subjects were young males possibly explains the higher mean value we obtained as compared with the one calculated from the observations of other investigators

TABLE 3

Determinations of pulmonary capacity in 50 healthy males

Absolute values

Pulmonary capacity	Mean	Standard deviation	Coefficient of variation	Variations
	liters	*liters*	*per cent*	*liters*
Total capacity	6 13±0 08*	0 82±0 06	13 3	4 42–7 86
Vital capacity	4 78±0 06	0 59±0 04	12 3	3 40–5 85
Complementary air	3 79±0 05	0 52±0 04	13 7	2 41–4 93
Reserve air	0 98±0 02	0 26±0 02	26 5	0 26–1 58
Mid capacity	2 34±0 05	0 49±0 03	20 9	1 09–3 38
Residual air	1 36±0 04	0 38±0 03	27 9	0 81–2 16

Relative values (Total capacity = 100 per cent)

	per cent	*per cent*	*per cent*	*per cent*
Vital capacity	78 0±0 41	4 3±0 29	5 5	68 4–85 5
Complementary air	61 9±0 51	5 3±0 36	8 5	51 7–78 9
Reserve air	16 2±0 39	4 1±0 28	25 3	5 0–30 3
Mid capacity	37 9±0 75	7 9±0 53	20 6	21 0–50 3
Residual air	22 0±0 41	4 3±0 29	19 5	14 5–31 6

Relative values (Vital capacity = 100 per cent)

	per cent	*per cent*	*per cent*	*per cent*
Complementary air	79 4±0 50	5 2±0 35	6 5	63 1–94 1
Reserve air	20 6±0 52	5 4±0 36	26 2	5 9–36 9
Mid capacity	49 1±0 85	8 9±0 60	18 1	25 0–70 4
Residual air	28 2±0 70	7 3±0 49	25 8	16 9–46 0

* Probable error

The *mid capacity* also shows wide fluctuations It varies between 1 09 and 3 38 liters, with a mean value of 2 34 ± 0 05 liters It shows a total variation of about 42 per cent from the mean Our results cannot be compared with those reported by other investigators on account of the difference in classification used Binger and Brow (3) in nine determinations of the mid capacity (called "functional residual air" in their report), which was measured in a manner analogous to ours, obtained, however, an average value of 2 43 liters in very close agreement with the mean value of this series

The volume of *residual air* varies markedly Its lowest and highest values in our series are 0 81 and 2 16 liters and the mean value is 1 36 ± 0 04 liter, a figure slightly lower than 1 50 liter which is the mean value of the cases previously reported

The *complementary* and *reserve volumes* show marked deviations from the corresponding mean values, especially the latter The diagram of Figure 1 illustrates graphically the mean values obtained for the total capacity and its subdivisions

Relative values It has been customary to express the values of the lung volume subdivisions as percentages of the total capacity This is the more valuable method of expressing the results, as one may readily see from an inspection of the diagram of Figure 2 The vital capacity mid
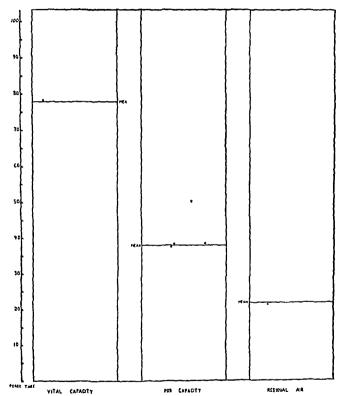
FIG 2 VITAL CAPACITY MID CAPACITY AND RESIDUAL AIR EXPRESSED IN PERCENTAGE OF THE TOTAL CAPACITY

The dots indicate 50 individual observations grouped about the mean values

capacity and residual air constitute more or less constant fractions of the total capacity in normal people, giving a sound basis for comparison with the possible variations of these ratios in pathological subjects In the case of the vital capacity we find that it has a mean value representing 78 0

± 0 41 per cent of the total volume   The standard deviation is only 4 3 per cent, so all variations actually come within 12 per cent of that mean value   It appears to indicate a rather constant relationship of vital capacity to residual air in constituting the total lung capacity   It is significant to find that the same ratio (Vital capacity/Total capacity) × 100 calculated from the cases reported in the literature has a mean value of 75 1 per cent, with a standard deviation of 4 2 per cent, that is a similar range of variability as found in our series   There is only one case (from the series of Lundsgaard and Van Slyke) in which this ratio was lower than 69 per cent

The residual air, expressed in percentage of the total capacity, varied in our series from 14 to 31 per cent, with a mean value of 22 0 ± 0 41 per cent   In 96 per cent of the cases it formed from 15 to 30 per cent of the total volume   The data in Table 4 show that the residual air varies more

TABLE 4

Comparison of residual air, vital capacity and total capacity

Ratio $\frac{\text{Residual air}}{\text{Total capacity}} \times 100$	Residual air	Vital capacity	Total capacity
per cent	*liters*	*liters*	*liters*
14–16	0 90	4 80	5 71
17	1 13	4 79	5 91
20	1 28	4 74	6 02
23	1 53	4 92	6 45
26	1 68	4 68	6 36
29	1 96	4 26	6 22

Correlation coefficients

Ratio $\frac{\text{Residual air}}{\text{Total capacity}}$ and residual air		+0 8740±0 0229*
Idem and vital capacity		+0 0164±0 0971
Idem and total capacity		+0 3898±0 0823

* Probable error

than the vital capacity   From the cases reported in the literature we obtained a mean value of 25 3 per cent in the ratio (Residual air/Total capacity) × 100, and in the recent series of Anthony (1) the average of eight determinations (also made in the recumbent position) was 23 2 per cent

The ratio (Mid capacity/Total capacity) × 100 has a mean value of 37 9 ± 0 75 per cent and a standard deviation of 7 9 per cent   In 96 per cent of the cases a ratio was between 30 and 50 per cent   The complementary and reserve airs, expressed as percentage of the total volume, show moderate variations in the case of the former, and very wide fluctuations in the percentage of the latter   The close correlation of the total capacity with the vital capacity, mid capacity and residual air may also be appreciated from the high and significant correlation coefficients calculated in our series and presented in Table 5

TABLE 5

Correlation between the different subdivisions

Capacities correlated	Correlation coefficient
Total capacity and vital capacity	$+0\,8950\pm0\,0188$*
Total capacity and complementary air	$+0\,7889\pm0\,0361$
Total capacity and reserve air	$+0\,4171\pm0\,0782$
Total capacity and mid capacity	$+0\,8020\pm0\,0337$
Total capacity and residual air	$+0\,7721\pm0\,0384$
Vital capacity and complementary air	$+0\,8836\pm0\,0209$
Vital capacity and reserve air	$+0\,4347\pm0\,0769$
Vital capacity and mid capacity	$+0\,5748\pm0\,0634$
Vital capacity and residual air	$+0\,4571\pm0\,0748$
Complementary air and mid capacity	$+0\,3296\pm0\,0850$
Complementary air and reserve air	$+0\,0195\pm0\,0951$
Complementary air and residual air	$+0\,4731\pm0\,0735$
Mid capacity and reserve air	$+0\,6191\pm0\,0587$
Mid capacity and residual air	$+0\,8202\pm0\,0310$
Residual air and reserve air	$+0\,1491\pm0\,0931$

* Probable error

Few investigators have expressed the values of the subdivisions of the pulmonary air as a percentage of the vital capacity There appears to be no special advantage in doing so, and perhaps it will be more convenient and simpler to use the total capacity as a basis for percentage estimations, rather than the vital capacity It is interesting to notice the rather constant relationship between complementary air and vital capacity, the former constituting about 80 per cent of the latter

Oxygen consumption and its relationship with ventilation per minute and vital capacity Efforts have been made by different investigators to correlate the oxygen consumption of the body with the ventilation in a given period of time in the hope of finding fixed ratios for normal people and possible deviations in pathological conditions Knipping and Moncrieff (5) have investigated the so called " ventilation equivalent for oxygen ' which may be calculated according to the formula (Minute volume respiration/O_2 used per minute) $\times 100$ They report observations in 54 normal subjects (31 males and 23 females) and obtained an average value of 2 44 liters for this ratio, with variations ranging between 1 68 and 3 70 liters In 19 cases of heart disease the results varied between 2 1 and 8 61 liters Close examination of their findings does not indicate any value in such a ratio as a measure of respiratory efficiency In 15 of our subjects in whom the determination of total capacity was done under basal conditions (rest and fasting) the calculation of that ratio gives an average of 2 66 with variations between 1 47 and 4 04 The coefficient of variation is very high and the correlation coefficient between oxygen consumption and ventilation per minute is not significant Anthony (1) has found in normal subjects a close correlation between the vital capacity and the calories utilized per 24 hours In the 15 cases already mentioned we obtained a very high coefficient of variation in the ratio (Vital capacity/O_2 consump

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STUDIES OF TOTAL PULMONARY CAPACITY AND ITS SUB-DIVISIONS II CORRELATION WITH PHYSICAL AND RADIOLOGICAL MEASUREMENTS

By ALBERTO HURTADO [1] and WALTER W FRAY

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In a previous paper (5) we have presented the results of 50 determinations of pulmonary capacity made in an equal number of healthy males It was demonstrated that the relative capacities (expressed in percentages of the total volume) fluctuated within narrow limits, but that there were wide variations in the absolute figures found for the total volume and its subdivisions This fact makes rather difficult the recognition of moderate and perhaps important, deviations in a given case and also prevents a clear understanding of any alterations in the relative values Thus a low ratio (Vital capacity/Total volume) $\times$ 100 may be caused by either increased residual air, low vital capacity, or a combination of both factors and the proper interpretation will be obtained only if normal values are available for comparison To standardize the procedure it appears to be necessary to find a correlation between the pulmonary capacity and certain bodily characteristics, so that from a knowledge of the latter it may be possible to predict the normal volumes If we have at our disposal reliable criteria to permit judgment of changes in absolute as well as in relative pulmonary capacity a clearer view of the underlying pathological physiology may be obtained A basis may also be provided for the proper classification and grouping of cases of respiratory inefficiency in which defective alveolar ventilation is an important factor

Although numerous investigations have been made to correlate the vital capacity to body or chest measurements there have been very few attempts to correlate these characteristics with the total pulmonary capacity or with any one of its subdivisions, other than vital capacity Lundsgaard and Van Slyke (7) in 1918 found in a few cases a definite correlation between the total capacity vital capacity mid capacity and residual air, and the so called "chest volume," calculated from the external measurement of the three diameters of the chest obtained at the end of full inspiration and expiration under resting conditions This investigation was later confirmed by Lundsgaard and Schierbeck (6) who found a similar relationship and also con-

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cluded that the degree of chest expansion (as determined by external measurements) affects the prediction of the corresponding pulmonary capacity But Binger and Brow (2) could not demonstrate such a correlation of pulmonary capacities, either with the external chest measurements, with the body height, or with the surface area, and suggested that the level of the diaphragm was the determining factor, since a long-chested type of individual with relaxed abdominal walls shows a greater total volume than the broad, deep-chested muscular type These investigators stated that the measurement of the area of the lung fields on radiographic films gives a fairly satisfactory means for predicting pulmonary capacity in normal individuals, although they did not prove so in the pneumonic patients whom they were then investigating No data were presented in their communication to support this conclusion Binger (1), while studying cases of cardiac decompensation, predicted the normal pulmonary capacity by assuming a vital capacity of 2 5 liters per square meter of body surface, and from this the other volumes were calculated on the basis of the rather fixed relative values of the main subdivisions of the total volume

We have made in our 50 cases a statistical study of the possible correlations of the pulmonary capacities with dimensions of the body and chest, and with radiological measurements It was also our aim in this study to gain helpful information concerning the comparative value of the methods for the determination of the degree of chest expansion, a factor seldom properly evaluated clinically but undoubtedly of importance in a consideration of respiratory mechanics To find the influence of the shape of the chest on the different subdivisions of pulmonary capacity was another purpose of the investigation

METHODS

The body height and weight were recorded in all cases, and the surface area calculated from the DuBois charts The following chest measurements were obtained in all subjects (without clothing) in the respiratory positions of full expiration, mid capacity (at the end of a normal expiration), and on full inspiration (a) *Chest circumference*—measured at the level of the nipple line, just below the pectoral prominences, (b) *Lateral diameter* (width), the distance between the midaxillary lines at the level of the nipple, (c) *Anteroposterior diameter* (depth), the distance from the midsternal line, also at the level of the nipples, to the vertebral column, and (d) *Longitudinal diameter* (height), a distance from the upper end of the sternum, at the mid-line, to the junction of the body with the xiphoid process of this same bone A pair of calipers (pelvimeter) was used in making the measurements

The external " chest volume " in the three respiratory positions was calculated (according to the Lundsgaard and Van Slyke technique) from the product of the diameters at the corresponding positions

Radiographs of the chest were obtained at the end of maximum expiration maximum inspiration, normal expiration and, in most instances, at the end of normal inspiration   These four exposures were obtained on two films in the following manner one of the films was first exposed at the end of maximum expiration, the exposure time being about three-quarters of that in routine radiography of the chest   A brass filter, 0 2 mm in thickness, was then placed over the chest at the level of the diaphragms, and a second exposure of the same length and character was made on the same film at the end of maximum inspiration   This film when developed shows the bony thorax and the diaphragm at both phases of respiration   The object in using the brass filter is to prevent over-exposure of the chest particularly of the diaphragm, during the second portion of the exposure   Much this same effect can be obtained without the use of the brass filter by making the length of exposure at expiration approximately five times that at inspiration   We found it more convenient to use the filter and at the same time obtained better definition of the upper portion of the thorax on inspiration   The principles of this technique have been explained by Thomas (1931) (8)

It is of the greatest importance that both exposures be made at the proper phase of respiration, i e after maximal effort, since the data obtained are used for the prediction of normal pulmonary capacity   We have found it convenient to use a signal (movement of fingers of one of the patient's hands) for timing the exposures

The second film was obtained in a similar manner, varying only the phase of respiration for the exposures to normal expiration and normal inspiration   It is extremely important that the exposure for expiration be made first, reversal of the order results in obliterating the shadow of the diaphragm at expiration

The films were obtained in the recumbent position   The individual was placed in the prone position on a table, the top of which had been modified to accommodate a tunnel in which the film cassette could be slipped without altering the position of the subject   The target film distance was six feet   The exposure time was approximately 1/20 second   The kilo-voltage was varied according to the thickness of the chest   The milliamperage remained constant at 200 milliamperes

Films obtained in this manner show the degree of expansion of the thorax and of the excursion of the diaphragm during maximal and quiet respiration   Because of the fact that such films do not impart any idea of the third dimension lateral films were tried during the early portion of the work in an attempt to define the anteroposterior diameter   The use of these were abandoned because of the difficulty in obtaining two photographic impressions of the upper portion of the chest on the same film

In determining the size of the chest two linear dimensions of the chest were used the transverse diameter and the height of the lung field (above

the level of the domes of the diaphragm) The transverse diameter was obtained at the level of the 9th interspace posteriorly, the measurement being taken from the inner aspects of the ribs The height of the lung fields was determined by measuring the vertical distance from the dome of the diaphragm to the plane of the first rib (EF expiration and GH inspiration, Figure 1)
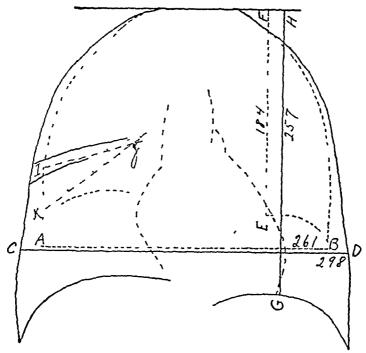
FIG 1 OUTLINES OF RADIOLOGICAL FILM WITH DOUBLE EXPOSURE TECHNIQUE (AT MAXIMUM EXPIRATION AND INSPIRATION)
Different measurements taken

These linear dimensions impart a very imperfect, or at least an incomplete, idea of the variation in chest size For this reason a planimeter, which recorded areas accurately up to 900 sq cm , was constructed The integrating wheel of this planimeter was approximately 7 5 cm in diameter, the tracing arm 50 cm in length, and the fixed arm 62 cm This instrument was calibrated by cutting circular grooves in a brass plate with diameters ranging from 10 to 40 cm The areas enclosed by these grooves were computed The planimeter was calibrated by comparing the readings on the scale after the point of the tracer arm completed the circle in the individual grooves with the respective areas as computed A graph was plotted from these determinations

With this instrument the areas of the lung fields were determined from the films obtained at the end of maximum and of normal expiration and inspiration The tracer arm was moved along the following course the

left axilla, left half of the diaphragm, right half of the diaphragm, right axilla, right apex and finally swept across the spine to the starting point at the left apex   The cardiac area, including the great vessels, was also obtained separately   The tracings obtained from films of a normal subject are illustrated in Figure 1   The area of the lung fields at maximum expiration was found to be 342 sq cm, and at maximum inspiration 571 sq cm   The difference represents the increase in area during maximal respiratory effort   Similarly, mensuration of the film taken at the end of normal expiration and inspiration yields data concerning the difference in area to be used in measuring reserve, complementary and tidal air, etc

The extent of motion of the ribs was measured by determining the angular displacement of ribs during maximum respiratory effort   The 7th rib (posterior portion) was usually selected because it could be seen best in a film taken at maximum expiration and inspiration   Lines were drawn representing the direction of the same rib at expiration and inspiration and the angle between them measured   In the normal subject illustrated, this angle (IJK) becomes 22°

Because of the curved course of the rib, its direction was determined by the slope of the outer two-thirds of its posterior portion

It was thought that a better approximate value of the size of the chest cavity could be obtained from combined external and radiological measurements   We have multiplied in all cases the area of the lung fields (the area of the heart included) expressed in square centimeters, by the corresponding anteroposterior diameter (depth) of the chest measured externally in centimeters   The result is designated in this paper as the " radiological chest volume "

CORRELATION OF THE PULMONARY CAPACITY WITH PHYSICAL AND RADIO LOGICAL MEASUREMENTS

The correlation of the total capacity and its main subdivisions with body and chest dimensions and with radiological measurements has been summarized in Table I   It is convenient to mention that correlation coefficients are significant only when they exceed the probable error multiplied by three and that correlation is proportionally better as coefficients approach the value of 1

In regard to the total capacity, we find that the highest correlation is obtained when the ' radiological chest volume ' (at maximum inspiration) is used   It is correlated significantly although in a lesser degree with the area of lung fields (also at maximum inspiration) and with the body height   There is no useful correlation between the total pulmonary capacity and external chest measurements, the correlation coefficients are either valueless or very low

The vital capacity is also better correlated with the " radiological chest volume '   Its correlation of $+0\,7174 \pm 0\,0467$ is the highest of the

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The vital capacity is also better correlated with the "radiological chest volume "   Its correlation of $+0\,7174 \pm 0\,0467$ is the highest of the

TABLE I

Correlation of pulmonary capacities with physical and radiological measurements

Characteristics correlated	Correlation coefficient
Total pulmonary capacity and body weight	$-0\ 1267 \pm 0\ 0944$*
Total pulmonary capacity and body height	$+0\ 5509 \pm 0\ 0661$
Total pulmonary capacity and body surface area	$+0\ 1644 \pm 0\ 0930$
Total pulmonary capacity and chest circumference (maximum inspiration)	$+0\ 1370 \pm 0\ 0944$
Total pulmonary capacity and chest volume (external) (maximum inspiration)	$+0\ 3271 \pm 0\ 0856$
Total pulmonary capacity and area of lung fields (maximum inspiration)	$+0\ 5848 \pm 0\ 0627$
Total pulmonary capacity and radiological chest volume (maximum inspiration)	$+0\ 6366 \pm 0\ 0566$
Vital capacity and body weight	$+0\ 1377 \pm 0\ 0930$
Vital capacity and body height	$+0\ 5452 \pm 0\ 0667$
Vital capacity and body surface area	$+0\ 2791 \pm 0\ 0876$
Vital capacity and chest circumference (maximum inspiration)	$+0\ 1591 \pm 0\ 0824$
Vital capacity and chest volume (external) (maximum inspiration)	$+0\ 3741 \pm 0\ 0816$
Vital capacity and area of lung fields (maximum inspiration)	$+0\ 6824 \pm 0\ 0509$
Vital capacity and radiological chest volume (maximum inspiration)	$+0\ 7174 \pm 0\ 0467$
Mid capacity and chest circumference (mid-position)	$-0\ 2361 \pm 0\ 0897$
Mid capacity and chest volume (external) (mid-position)	$-0\ 0554 \pm 0\ 0957$
Mid capacity and area of lung fields (mid-position)	$+0\ 5432 \pm 0\ 0674$
Mid capacity and radiological chest volume (mid-position)	$+0\ 4437 \pm 0\ 0769$
Residual air and chest circumference (maximum expiration)	$-0\ 0737 \pm 0\ 0944$
Residual air and chest volume (external) (maximum expiration)	$+0\ 1065 \pm 0\ 0937$
Residual air and area of lung fields (maximum expiration)	$+0\ 3707 \pm 0\ 0823$
Residual air and radiological chest volume (maximum expiration)	$+0\ 3826 \pm 0\ 0809$

* Probable error

series, and consequently will be advantageously taken as the basis for the prediction of the normal pulmonary capacity in a given case It is interesting to observe the almost valueless correlation between the vital capacity and the body surface area, a rather surprising finding as it has been widely accepted in clinics, following the work of West (9) in 1920 He found that this volume is better related to body surface area than to any other physical dimension, normal men having a vital capacity of about 2 5 liters per square meter From the present observations and from previous experience, we think that there are wide variations in this relationship, it is usually found only when there is a normal balance between height and weight, and is not obtained in subjects who are either over or under weight This has been so frequently observed in various clinics that it has limited the usefulness of the vital capacity determinations on the basis of body surface area, unless marked alterations are found

The scatter of our fifty observations around the lines of correlation when the total capacity, the vital capacity and the different bodily thoracic and radiological measurements are compared, may be appreciated from a study of the Figures 2 and 3 It is plainly evident that the best correlation is

FIG 2   CORRELATION BETWEEN THE TOTAL PULMONARY CAPACITY AND
PHYSICAL AND RADIOLOGICAL MEASUREMENTS

Lines represent ideal correlations (from the regression equations)   Dots
represent individual observations

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found in the case of the " radiological volume " and (a) the total capacity (Fig 2) and (b) the vital capacity (Fig 3)

When we come to the mid capacity we find a less perfect correlation with radiological data and a complete lack of relationship with external chest measurements It is correlated best with the area of lung fields (radiological exposure at the end of a normal expiration), a fact worth taking into account in investigations where this subdivision of total volume is the only one that was determined and where knowing its normal value is desirable [2] The residual air is in very low correlation with radiological measurements, and in no correlation with external chest measurements

From these observations it appears that we have been unable to confirm the early work of Lundsgaard and Van Slyke, and Lundsgaard and Schierbeck in demonstrating a correlation between the total capacity and its main subdivisions with the " chest volume " calculated from the three external diameters of the chest This conclusion could have been predicted for it is evident that although the lateral and anteroposterior diameters may represent the dimensions of the chest in these planes the measurement of its height is not identical with the length of the sternum, and cannot indicate the level of the diaphragm, which varies widely from and independently of the sternal length The " chest volume " calculated from these measurements yields consequently a doubtful value of the true volume of the chest cavity Theoretically, it appeared to be a sounder assumption that multiplying the area of the lung fields measured in radiological films and the anteroposterior diameter of the chest, determined externally, would secure a value more accurately representative of the true size of the chest cavity This assumption has been verified, for in this way a better correlation has been found between the volume of the lungs and that of the chest

PREDICTION OF THE NORMAL CAPACITY OF THE LUNGS

It has now been shown that in the total series of correlations between the total pulmonary capacity and its components and the physical and radiological measurements the highest and most significant correlation coefficient has been observed between the vital capacity and the " radiological chest volume " This fact indicates that if the chest volume is known in a given case the vital capacity may be predicted with a fair degree of accuracy by means of the regression formula derived from the correlation coefficient [3] Other fractions of the total capacity may be inferred from the percentage values which the vital capacity, the mid capac-

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[2] The regression formula derived from the correlation coefficient between the mid capacity volume and the area of lung fields (also at the same respiratory position) is as follows

Mid capacity (liters) = (area of lung fields in cms $\times 0 0042$) $+ 0 33$

[3] The regression formula is

Vital capacity (liters) = (radiological chest volume (liters) $\times 0 24$) $+ 1 22$

anteroposterior diameters vary widely, it is impossible therefore in a given case to justify the use of these measurements It may be said in a general way that a circumferential expansion of less than 6 cm is to be considered as reduced No correlation could be found between pulmonary capacity and chest expansion determined by external measurements

If on the other hand we measure from radiological films the areas of the lung fields at maximum expiration and inspiration the relation between the two varies within definite limits The ratio (Area at maximum expiration/Area at maximum inspiration) $\times$ 100 has, in our series, a mean value of 62 2 with a total deviation from the mean of less than 15 per cent It appears that this ratio may, therefore be used as a means of appreciating the degree of chest expansion If it is higher than 72 0 we may suspect that a definite reduction in excursion has taken place This ratio it must be remembered although it takes into consideration the diaphragmatic and lateral expansion of the chest neglects expansion in the anteroposterior diameter But since as we have found, the mean values of the lateral and anteroposterior chest expansion are practically the same, the error will be more or less constant As a matter of fact, we have found, in a group of cases of chronic pulmonary disease this similarity of expansion in the two planes

The ratio (Area at maximum expiration/Area at maximum inspiration) $\times$ 100 is higher in people with a long narrow chest and is chiefly altered by changes in the diaphragmatic excursion It is interesting to find a relationship between this ratio and the relative values of the pulmonary capacity In this series of normal cases the correlation coefficient between the ratios (Area at maximum expiration/Area at maximum inspiration) $\times$ 100 and (Residual air/Total volume) $\times$ 100 is significant $+$ 0 4015 $\pm$ 0 0796 indicating that alterations in one ratio tend to be accompanied by similar and proportional changes in the other We will show in an other communication that this relationship is considerably more evident in pathological cases in which when expansion is diminished the residual air is high This relationship between chest expansion and relative pulmonary capacity suggests that correction of the latter is necessary if expansion is diminished A consequence of correcting such a value may be the neglect of a pathological factor so that a value significantly altered in fact may appear to be normal It will be more helpful to have both ratios for the proper interpretation of a case rather than to modify one by means of the other

Additional information concerning chest expansion may be obtained from a study of other measurements taken from the same radiological film The diaphragmatic excursion the lateral expansion of the chest and the degree of movement of the rib although showing considerable normal variations may be considered reduced when they move less than 4 cm 2 cm and 12° respectively

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SHAPE OF THE CHEST AND LUNG VOLUME

In all of our 50 cases two different indices have been calculated to determine the relationship between the shape of the chest and changes in pulmonary capacity and chest expansion. The index (Depth/Width) $\times$ 100 will indicate whether the chest is of the rounded or flat type. There are wide variations in this index from 58 to 84 with a mean value of 68 5 (Table III). In comparing this index with the different absolute and rela-

TABLE III
Shape of the chest

Indices	Mean	Standard deviation	Coefficient of variation	Variations
			per cent	
$\dfrac{\text{Depth}}{\text{Width}} \times 100$	68 5±0 55*	5 8±0 39*	5 4	58 3–84 4
$\dfrac{\text{Height†}}{\text{Width}} \times 100$	70 8±0 71	7 3±0 49	10 3	50–84

* Probable error
† This measurement represents the average height of both sides of the diaphragm (at mid capacity) as determined on the radiological films
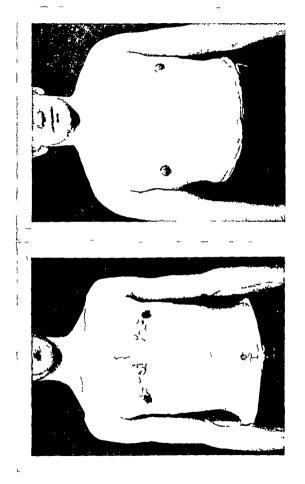
tive volumes, we have not found a significant correlation to indicate that variations in the pulmonary capacity may be attributed to this factor There was no correlation, either, between this index and the degree of chest expansion, except that the movement of the ribs is less marked when the chest index is high that is to say in rounded chests

The index (Height/Width) $\times$ 100 (both diameters being measured on x-ray films taken at mid capacity distinguishes two types of chest broad, muscular ones with a high diaphragm (hypersthenic type), and slender and long narrow ones with a low diaphragm (asthenic type) In the former the index is low and in the latter high We have found that the shape or type of the chest from this point of view has a definite influence upon the pulmonary capacity, and that this is reflected chiefly in changes in the volume of reserve air In our series of normal males, it was not an uncommon observation to find a rather low vital capacity (as compared with the calculated value) in the hypersthenic type of individual due to the small volume of reserve air The correlation coefficient between the index (Height/Width) $\times$ 100 and the reserve air was $+0.4133$ ± 0.0803 which is significant In the three cases in which the lowest indices were obtained (50 52 and 58) the observed reserve air was 0 26, 0 50 and 0 38 liters respectively

The radiological chest volume was considerably larger in those individuals with long narrow chests and low diaphragms They exhibited correspondingly the highest total pulmonary capacity This fact stands in

FIG 5 ASTHENIC (LEFT) AND HYPERSTHENIC TYPES OF CHEST

The latter has a smaller total volume and a marked decrease in the volume of reserve air as compared with the asthenic chest The diaphragmatic level is much higher in the hypersthenic chest

sharp contrast with what one might judge clinically, because men with broad muscular and apparently big chest cavities are regarded as having greater pulmonary volumes compared with the other type. The reverse is, however, the case, indicating the importance of taking into consideration the diaphragmatic level in calculating the pulmonary capacity in a given case

Comparing the chest expansion in hypersthenic individuals (broad muscular chests with high diaphragms) we did not find any significant relationship between this and the ratio (Area at maximum expiration/Area at maximum inspiration) $\times$ 100 and the diaphragmatic excursion. There was however, almost no lateral expansion of the chest during the determination of the reserve air (a forced expiration from the mid capacity level) in contrast with other cases in which an appreciable expansion was noticed and measured

SUMMARY AND CONCLUSIONS

In a preceding paper (5) have been presented the values of pulmonary capacity found in observations made on 50 healthy males. In the present communication we have discussed the correlation between the total capacity and its main subdivisions with height, weight, surface area and external and radiological measurements of the thorax. It has been shown that they may be best calculated in a given case from the so-called " radiological chest volume ". The method of calculation has been fully presented, comparison of the observed and the calculated volumes shows a very close correspondence. The application of these observations permits one to appreciate pathological deviations

Studies have been made of the normal variability in the degree of expansion of the chest, which is best exhibited in measurements of the chest film taken by means of a standard technique which has been described. The same film, together with the measurement of the anteroposterior diameter of the chest at maximum inspiration furnishes all necessary information for the calculation of a given pulmonary capacity if correlation coefficients and regression formulae are used

The influence of the shape of the chest on the pulmonary capacity and on the degree of expansion of the chest has also been investigated

The observations presented lead to the following conclusions

1 When the total pulmonary capacity and its main subdivisions are calculated on the basis of the ' radiological chest volume " at maximum inspiration the following deviations in the observed values (as compared with the calculated ones) are considered to be significant a difference of over 15 per cent in the total pulmonary capacity and vital capacity, and of 30 per cent and 40 per cent in the mid capacity and residual air respectively

2 It the ratio (Area at maximum expiration Area at maximum inspiration) $\times$ 100 is higher than 72.0 a reduction in chest expansion is

indicated   Further evidence of deficient expansion is obtained if the diaphragmatic excursion the lateral expansion and the degree of move ment of the rib are found to be less than 4 cm 2 cm and 12° respectively

3 There is a certain correlation between the degree of chest expansion (as appreciated by the ratio mentioned) and the relative proportions of subdivisions of the pulmonary capacity   Deficient expansion tends to be accompanied by a higher percentage of the residual air in relation to the total capacity

4 There is a relationship between the shape of the chest and the capac it} of the lungs   Individuals with broad muscular chests and high dia phragms (hypersthenic type) usually present low volumes of reserve air as compared with long and narrow chested individuals with low dia phragms (asthenic type)   The latter have larger thoracic capacity and consequently larger pulmonary capacity

5 From the observations made on normal males it is possible to de tect pathological changes in the absolute and relative pulmonary capacity in a given case   The importance of recognizing such alterations is obvious

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STUDIES OF TOTAL PULMONARY CAPACITY AND ITS SUB-DIVISIONS III CHANGES WITH BODY POSTURE

By ALBERTO HURTADO[1] and WALTER W. FRAY

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It has been known for many years that the posture of the body has an influence on pulmonary capacity. More than 25 years ago Bohr (3) and Plesch (10) found that the residual air increases in the recumbent position. Christie and Beams (6) in 1922 observed a reduction of 5.5 per cent in the vital capacity in the latter position and Wilson (11) a few years later demonstrated that this is brought about by a marked reduction in the reserve air. Binger and Brow (2) in 1924 found a considerable decrease in the functional residual air (mid capacity) with subjects flat in bed. The average reduction in nine cases was 0.80 liters. In recent years Anthony (1) reported practically no change in the volume of residual air depending on different positions of the body but confirmed the previous finding that there is marked reduction in the reserve air in the recumbent position and pointed out that the volume of complementary air changes in the opposite direction, it increases on assuming this position. Calhoun Cullen and Harrison et al (5) in 1931 found similar influences due to the position of the body on the pulmonary capacity.

We have shown in a previous paper (8) that there is a definite correlation between the total capacity and its subdivisions and the size of the chest cavity. It will be interesting therefore to discover whether changes in position are also accompanied by similar and proportional alterations in size and expansion of the chest. Hamilton and Morgan (7) in a recent communication reported that in five normal subjects there was no change in the volume of residual air in the recumbent position but they observed a moderate decrease in the vital capacity and consequently a corresponding diminution in the total capacity when lying was compared with the sitting posture. These investigators measuring the lateral and anteroposterior diameters of the chest found a moderate increase when the subjects were recumbent but failed to find consistent changes in the level of the diaphragm. From their observations they concluded that the chest cavity was larger when the body is recumbent and that the reduction in total volume

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[1] Travelling Fellow of the Rockefeller Foundation

and vital capacity in this posture possibly is brought about by accumulation of blood in the pulmonary circuit

There is evidence in the literature to show that when recumbent there is diminution in the size of the cavity of the chest   Briscoe (4) from careful observations concluded that in this position there is a considerable reduction and that this is affected chiefly by an elevation of the diaphragm, while the lateral and anteroposterior diameters of the chest show very slight increase   Livingstone (9) in 1928 reported that in normal individuals the capacity of the chest is least in the supine posture and that this corresponds with a higher level of the diaphragm and with practically no change in the diameters of the thorax

To study this matter further, we have made observations of 10 healthy males (average age 23 years) regarding the influence of posture on the capacity of the lungs and the size and expansion of the chest   The determinations of pulmonary capacity in the sitting and recumbent positions were made with a few minutes interval   Radiographs of the chest were obtained at maximum and at normal expiration and inspiration   The methods and technique employed in these observations and the different measurements obtained from the radiographs have already been described (8)

CHANGES IN PULMONARY CAPACITY WITH BODY POSTURE

The variations found (Table I) are interesting and, in general agree with the work of previous investigators   When subjects assume the recumbent position there is as compared with the sitting posture, a moderate reduction in the total capacity vital capacity, and residual air (the average diminutions are 9 0 6 6 and 14 5 per cent respectively) with more marked changes in the reserve air and mid capacity (28 2 and 41 7 per cent)   The only volume which shows an alteration in the opposite direction is the complementary air   Its increase (11 1 per cent) is, however, proportionally lower than the decrease in the reserve air, so that the vital capacity is nevertheless reduced

In the course of our investigation we also determined the vital capacity of 32 healthy males in the erect and lying positions   The results of these observations closely agree with the corresponding changes found in the ten cases   The average vital capacity in the erect posture was 5 06 liters while in the lying position it was 4 77 liters a difference of 0 29 liters a reduction of 5 7 per cent

The alterations in the absolute values of some of the subdivisions on assuming the recumbent posture are of course reflected in their relative values because the total volume is proportionally less altered   There are very slight changes in the ratios   (Vital capacity/Total volume) and (Residual air/Total volume) but on the other hand there are marked reductions in the ratios of reserve air and mid capacity to the total volume

TABLE I

Changes in pulmonary capacity according to posture (mean of 10 observations)

Determinations	Means		Variation	
	Sitting	Lying		
	liters	*liters*	*per cent*	*liters*
Total pulmonary capacity	6 64±0 18	6 04±0 19*	−9 0	0 60
Vital capacity	4 99±0 12	4 66±0 14	−6 6	0 33
Complementary air	3 32±0 10	3 69±0 12	+11 1	0 37
Reserve air	1 68±0 07	0 98±0 06	−41 7	0 70
Mid capacity	3 33±0 14	2 39±0 11	−28 2	0 94
Residual air	1 65±0 10	1 41±0 09	−14 5	0 24

Ratios	per cent	per cent		
Vital capacity/Total volume	75 4±1 17	76 9±1 01	+1 9	
Complementary/Total volume	49 6±1 15	60 8±0 96	+22 5	
Reserve/Total volume	25 3±0 83	16 2±0 96	−35 9	
Mid capacity/Total volume	49 9±1 15	39 2±0 95	−21 4	
Residual/Total volume	24 5±1 06	23 0±1 13	−6 1	
Complementary/Vital capacity	64 8±1 95	78 8±1 19	+21 5	
Reserve/Vital capacity	33 5±1 06	21 0±1 12	−37 3	
Mid capacity/Vital capacity	66 7±2 47	51 3±1 78	−23 0	
Residual/Vital capacity	33 1±2 05	30 4±1 85	−8 1	

* Probable error

The complementary air increases and therefore its ratio is increased   The complementary air makes up a bigger part of the vital capacity and the reserve air the other component is correspondingly reduced

The changes just mentioned in the absolute and relative values of the pulmonary capacity and its subdivisions according to posture indicate the necessity of making all determinations under similar and standard conditions to permit proper comparison   This fact has often been overlooked in the past

INFLUENCE OF POSTURE ON SIZE AND EXPANSION OF THE CHEST

The different measurements obtained from the radiological films taken in the sitting and recumbent postures are summarized in Table II   Considering first the changes occurring in the size of the chest it will be noted that when recumbent the level of the diaphragm is distinctly higher, and that this is more marked at mid capacity (after a normal expiration)   It is also higher at maximum expiration and inspiration but to a much smaller degree   The actual measurements of the height of the right and left sides of the diaphragm show that at mid capacity they are 5 6 and 4 1 cm higher than in the sitting position   The higher level attained is slightly more marked in the right diaphragm

TABLE II

Changes in size and expansion of the chest with body posture

(From radiological observations—mean of 10 cases)

Measurement	Means		Variations		Measurement	Means		Variations	
	Sitting	Lying	per cent	units		Sitting	Lying	per cent	units
	units	units		units		units	units		units
Height of diaphragm—right cm At full expiration — left	18 7±0 56* 20 5±0 53	17 1±0 17 19 2±0 51	-8 5 -6 3	-1 6 -1 3	**Excursion of diaphragms** Reserve—right cm left	5 0±0 37 3 9±0 32	0 7 ±0 12 0 83±0 11	-86 0 -78 1	-1 3 -2 97
At mid capacity—right cm left	22 9±0 65 23 6±0 71	17 3±0 13 19 5±1 26	-24 1 -17 3	-5 6 -4 1	Complemental—right cm left	1 5±0 25 2 1±0 28	6 1 ±0 25 6 4 ±0 28	+326 +166	+1 9 +4 0
At full inspiration—right cm left	24 9±0 50 26 7±0 50	23 6±0 46 25 8±0 16	-1 8 -3 3	-1 2 -0 9	Total—right cm left	6 0±0 21 6 1±0 19	6 5 ±0 21 6 6 ±0 21	+8 3 +8 2	+0 5 +0 5
Width of chest At full expiration cm At mid capacity cm At full inspiration cm	26 9±0 18 27 6±0 19 30 7±0 29	27 3±0 28 28 1±0 31 30 3±0 24	+1 1 +2 9 -1 3	+0 4 +0 8 -0 1	**Expansion in chest width** Reserve cm Complemental cm Total cm	0 7±0 09 3 4±0 19 3 8±0 18	1 2 ±0 15 1 7 ±0 19 3 0 ±0 18	+71 1 -50 0 -21 0	+0 5 -1 7 -0 8
Area of lung fields At full expiration cm² At mid capacity cm² At full inspiration cm² Cardiac area cm²	128±15 5 555±20 6 671±17 1 157±3 17	398±18 0 430±14 0 639±16 7 155±2 96	-7 0 -22 4 -1 7 -1 2	-30 -125 -32 -2	**Changes in area of lung field** Reserve cm² Complemental cm² Total cm²	110±0 21 104±8 21 243±9 55	15±2 75 202±10 1 241±9 39	-67 8 +91 2 -0 8	-95 +94 -2
					Rib rotation degrees	20±0 99	18±1 24	-10	-2
					$\dfrac{\text{Area at maximum expiration}}{\text{Area at maximum inspiration}} \times 100$ Ratio	63 6±1 24	62 1±1 11	-2 3	-1 5

* Probable error

It is interesting to see that the lateral diameter of the chest shows a very slight change, less than one centimeter, when the posture is varied from sitting to recumbency We have not measured the anteroposterior diameter but from other investigations already cited it probably shows no change

The size of the areas of lung fields shows similar alterations   At mid capacity it is reduced 22 4 per cent   At maximum expiration and inspiration the areas are slightly smaller although the decrease is only 7 0 and 4 7 per cent respectively

From these observations it appears that the alterations in the size of the chest are of similar character to the variations in pulmonary capacity We have seen that the volume of mid capacity is most markedly reduced (as compared with the total volume and residual air) and that similar alteration occurs in the radiological area of the lungs at this respiratory position   We have found that the decrease in pulmonary capacity is some-what more marked however than the changes in the radiological area of the lungs   The total volume is reduced 9 0 per cent while the area at full inspiration is only 7 0 per cent smaller   The mid capacity decreases 28 2 per cent while the corresponding area shows a reduction of 22 4 per cent and finally the residual air decreases 14 5 per cent and the area at maximum expiration has diminished only 4 7 per cent in size   Does this mean that part of the alveolar space is encroached upon by the increased size of the capillary bed? This explanation may be correct as has often been suggested but it is worth emphasizing that we have not found a marked discrepancy between the changes in pulmonary capacity and chest size

Comparison of the expansion of the chest in the sitting and recumbent positions is interesting   We will consider first the diaphragmatic excursion   While recumbent there is a very slight increase in the maximum excursion but the difference is less than a centimeter   The movement of the diaphragm during the determination of the reserve air is much decreased   This is evident on both sides although slightly more on the right While sitting   the reserve excursion of the right diaphragm is 5 0 cm and of the left 3 0 cm   and it decreases to 0 7 and 1 0 respectively in recumbency   On the other hand the 'complemental" diaphragmatic excursion (during a forced inspiration starting from the mid capacity) shows a very striking increase in recumbency

The measurements of the areas of the lungs on a radiograph at different respiratory positions (at maximum expiration and inspiration, and after a normal expiration and inspiration) indicate the changes in the size of these areas during the determination of the several subdivisions of the total volume (vital capacity   reserve air   complementary and tidal air)   By comparing these differences in area in the sitting and recumbent positions we find that in the latter posture there is relatively a small variation in

SUMMARY AND CONCLUSIONS

The effects of posture upon pulmonary capacity and the size and expansion of the chest have been observed in 10 healthy males by comparing measurements made in the recumbent and sitting postures

It has been found that when recumbent there are slight decreases in the total volume, the vital capacity and the residual air but the reserve air decreases markedly   On the other hand there is a marked increase in the volume of the complementary air

Similar and parallel decreases although proportionally less marked have been demonstrated to occur in the size of the chest   This diminution is most marked at mid capacity and it is caused by an upward displacement of the diaphragm

An analysis of the expansion of the chest in both sitting and recumbent positions shows also parallel changes   In the latter posture the diaphragmatic excursion and the change in the area of the projection of the lungs corresponding to the reserve air are considerably reduced while the reverse is true in relation to the complementary air

These observations furnish additional data regarding the close correlation existing between the pulmonary capacity and the size and expansion of the chest and indicate the necessity for adopting a standard posture when investigations of this nature are made

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STUDIES OF TOTAL PULMONARY CAPACITY AND ITS SUB-DIVISIONS IV PRELIMINARY OBSERVATIONS ON CASES OF PULMONARY EMPHYSEMA AND OF PNEUMOCONIOSIS

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There has been no practical method of estimating in a quantitative way the degree of respiratory inefficiency in cases of chronic pulmonary disease Although many observations have been made in regard to their underlying pathological anatomy and physiology there has not been any serious attempt to correlate such observations with the clinical condition especially with respect to adaptation to the increased respiratory demands on physical activity Investigators have usually been concerned with circulatory and internal respiratory functions In previous papers (3) (4) we have presented the results of our measurements of capacity of the lungs and of chest expansion in an attempt to establish the normal variation thus enabling one to distinguish pathological deviations In the present communication are presented similar observations in cases of chronic pulmonary diseases

METHODS AND CASES STUDIED

A complete description of the methods used in measuring the total pulmonary air and its subdivisions and in the prediction of their normal values in a given case has been discussed in previous papers (3) (4) Measurements of pulmonary ventilation during physical activity have also been made using a modified bicycle ergometer and a 100 liter Tissot spirometer adapted to the graphic registration of the respirations In some cases arterial blood was obtained and its oxygen saturation and carbon dioxide content determined by the manometric method of Van Slyke (7) We have studied 15 cases, 9 cases of pulmonary emphysema and 6 cases of pneumoconiosis Several patients presented evidence of heart disease but no evidence of heart failure was present at the time of these observations In each group there was considerable variation in the extent of pulmonary disease This was an important consideration as one of the main purposes of this investigation was to correlate the findings with the degree of functional respiratory disability A summary of each case is given below

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Case 1 Male 41 years Chronic cough for 27 years Severe asthmatic attacks during the last few years *Very severe dyspnea on physical activity* No cardiac symptoms
Chest markedly emphysematous Heart normal Radiograph showed increased linear markings and emphysema
Diagnosis Chronic bronchitis Asthma Pulmonary emphysema

Case 2 Male 54 years Chronic cough and asthmatic attacks for 20 years *Severe dyspnea on physical activity* No cardiac symptoms
Chest markedly emphysematous Heart normal Electrocardiogram normal Radiograph showed increased linear markings
Diagnosis Chronic bronchitis Asthma Pulmonary emphysema

Case 3 Male 51 years Chronic cough expectoration and asthmatic attacks for two years *Severe dyspnea on physical activity*
No cardiac symptoms
Chest very emphysematous Radiograph showed slight left sided cardiac enlargement and increased lung markings Electrocardiogram presented evidence of myocardial damage
Diagnosis Chronic bronchitis Asthma Pulmonary emphysema Chronic myocarditis (compensated)

Case 4 Male 41 years Chronic cough and asthmatic attacks for 18 years *Only slight dyspnea on physical activity* No cardiac symptoms
Chest not emphysematous in appearance Hyperresonance on percussion over precordial and hepatic areas Electrocardiogram normal Radiograph of the chest showed prominence of the lung marking and of the pulmonary artery suggesting early fibrosis and pulmonic arteriosclerosis
Diagnosis Chronic bronchitis Asthma Pulmonary emphysema Pulmonic arteriosclerosis

Case 5 Male 60 years Dry cough for many years *Moderate dyspnea on physical activity* Frequent attacks of precordial pain for two or three years
Chest definitely emphysematous in appearance Heart enlarged to the left General arteriosclerosis
Diagnosis Arteriosclerotic heart disease Pulmonary emphysema

Case 6 Male 26 years Asthmatic attacks since childhood *No dyspnea on physical activity*
Chest not emphysematous Heart normal Radiograph of chest showed increased linear markings and evidence of an early emphysema
Diagnosis Asthma Pulmonary emphysema

Case 7 Male 41 years Attacks of precordial pain for seven years During the last two years attacks of unconsciousness accompanied with convulsions *No dyspnea on exertion*
Chest markedly emphysematous Heart enlarged to the left Radiograph of the lungs showed pulmonary fibrosis and emphysema
Diagnosis Coronary heart disease Question of Adams-Stokes syndrome Pulmonary emphysema

Case 8 Male 50 years *Slight dyspnea on exertion*
Chest does not appear emphysematous Heart enlarged to the left Electrocardiogram showed left ventricular preponderance and myocardial damage Radiograph of the chest suggested pulmonary emphysema pleural thickening and cardiac hypertrophy
Diagnosis Arteriosclerotic heart disease Pulmonary emphysema

Case 9   Male 61 years   Chronic cough for three years   *Very severe dys
pnea* on physical activity   No cardiac symptoms
Chest markedly emphysematous   Heart enlarged to the left   Elec
trocardiogram showed an intraventricular conduction defect   Radio
graph of the chest revealed a marked degree of emphysema   enlarge-
ment of the left ventricle and increased linear markings
Diagnosis Chronic bronchitis   Arteriosclerotic heart disease   Pul
monary emphysema

Case 10   Male 48 years   Had worked in sand blasting for 6 years   Chronic
cough for four years   *Severe dyspnea on physical acti ity*   No car
diac symptoms
Chest emphysematous   Heart not enlarged   Electrocardiogram
gave questionable evidence of myocardial damage   Radiograph of
the chest showed marked increase in linear markings and typical
appearance of pneumoconiosis and emphysema at both bases
Diagnosis Pneumoconiosis   Pulmonary fibrosis

Case 11   Male 35 years   Had worked at sand blasting for 5 years   Chronic
cough for two years   *Moderate dyspnea on exertion*   Able to carry
on light work   Complains of palpitation on physical activity
Chest did not appear emphysematous   Heart not enlarged   Radio
graph of the chest showed characteristic changes of pneumoconiosis
pulmonary fibrosis and emphysema   Electrocardiogram normal
Diagnosis Pneumoconiosis   Pulmonary fibrosis

Case 12   Male 46 years   Had worked at sand blasting for 2 years   Slight
cough during the last year   *Moderate dyspnea on physical activity*
No cardiac symptoms
Chest had normal appearance   Heart not enlarged   Electrocardio
gram normal   Radiograph of the chest suggested pneumoconiosis
and pulmonary emphysema
Diagnosis Pneumoconiosis   Pulmonary fibrosis

Case 13   Male 42 years   Had been exposed to inhalation of siliceous dust for
18 months   *Moderate dyspnea on exertion*   No other symptoms
Chest had emphysematous appearance   Heart slightly enlarged to
the left   Electrocardiogram indicated left ventricular preponderance
Radiograph of the chest showed changes consistent with pneumocon
osis (Early second stage)
Diagnosis Pneumoconiosis   Pulmonary fibrosis   Myocardial dis
ease (compensated)

Case 14   Male 42 years   Had worked at sand blasting for 8 months   Chronic
cough during the last two years   He thought that in the last two
months he had had *slight dyspnea on physical acti ity*   No other
symptoms
Chest normal   Heart not enlarged   Radiograph of the chest showed
increase in size of the hila and increased markings but no mottling
Appearance of emphysema in both bases
Diagnosis Question of pneumoconiosis   Question of pulmonary
fibrosis

Case 15   Male 56 years   Had been severely exposed to inhalation of siliceous
dust 7 years   Hemoptysis frequent cough and *severe dyspnea on
exertion*   Edema of lower extremities
Chest markedly emphysematous   Heart greatly enlarged   Electro
cardiogram showed right ventricular preponderance   Radiograph of

the lungs revealed a diffuse soft mottling   Pulmonary artery promi
nent   The total volume of the blood was increased
Diagnosis Pneumoconiosis   Pulmonary fibrosis   Sclerosis of the
pulmonary artery   Myocardial disease   Secondary polycythemia
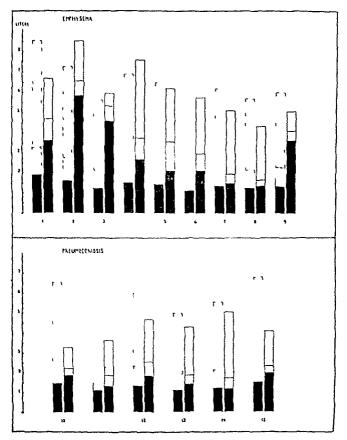
Observations on pulmonary capacity

Pulmonary emphysema   The calculated and the observed values for
the total pulmonary air and its main subdivisions in the nine subjects
having pulmonary emphysema are presented in Table 1, and are shown in
Figure 1   In these cases the total capacity of the lungs which was ob-

TABLE 1

Comparison of observed pulmonary capacity with calculated normal values in emphysema and in pneumoconiosis

Case num ber	Total capacity			Vital capacity			Mid capacity			Residual air		
	Calcu lated	Ob served	Dif ference	Calcu lated	Ob served	Dif ference	Calcu lated	Ob served	Dif ference	Calcu lated	Ob served	Dif ference
	liters	liters	per cent	liters	liters	per cent	liters	liters	per cent	liters	liters	per cent
Pulmonary emphysema												
1	8 53	6 64	−22 2	6 66	3 05	−54 2	3 24	4 67	+ 44 1	1 89	3 59	+ 90 0
2	7 25	8 52	+17 5	5 66	2 70	−52 3	2 75	6 56	+138 5	1 59	5 82	+266 0
3	5 62	5 96	+ 6 0	4 39	1 42	−67 7	2 13	5 32	+149 0	1 23	4 54	+269 1
4	6 89	7 59	+10 1	5 38	4 95	− 8 0	2 62	3 72	+ 41 9	1 51	2 64	+ 74 8
5	6 47	6 18	− 4 5	5 05	4 00	−20 8	2 46	3 56	+ 44 7	1 42	2 18	+ 53 5
6	5 02	5 76	+14 7	3 92	3 64	− 7 2	1 91	2 96	+ 54 9	1 10	2 12	+ 92 7
	6 20	5 08	−18 1	4 84	3 60	−25 7	2 36	1 96	− 17 0	1 36	1 48	+ 8 5
	5 70	4 32	−24 3	4 45	2 98	−33 1	2 17	1 66	− 23 5	1 25	1 34	+ 7 2
	5 98	5 04	−15 8	4 67	1 44	−69 2	2 27	4 08	+ 79 7	1 31	3 60	+174 8

FIG. 1 CALCULATED AND OBSERVED PULMONARY CAPACITY IN CASES OF
PULMONARY EMPHYSEMA AND PNEUMOCONIOSIS

Each case is represented by two columns. The one on the left with broken
lines is the predicted value, and the right column is the observed volume.

In black, residual air. Transverse line dividing the white area (vital capac-
ity) is the mid capacity level.
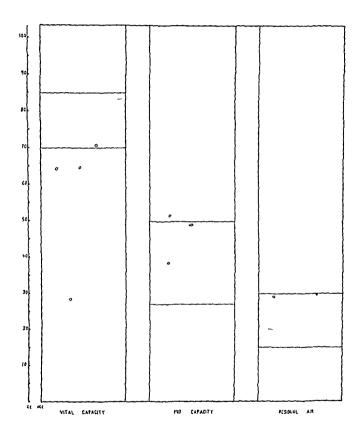
Fig 2   RELATIVE PULMONARY CAPACITIES (TOTAL CAPACITY = 100 PER CENT) IN CASES OF PULMONARY EMPHYSEMA AND PNEUMOCONIOSIS

The shaded areas represent limits of normal variations   Black dots are cases of pulmonary emphysema   circles are cases of pneumoconiosis

These alterations in the absolute values of the pulmonary airs are reflected in the relative values   That this is so may be appreciated by a study of Table 2   The vital capacity is reduced and the residual air cor-

TABLE 2

Relative values of subdivisions of pulmonary capacity in emphysema and pneumoconiosis

Case number	$\dfrac{\text{Vital capacity}}{\text{Total volume}} \times 100$		$\dfrac{\text{Residual air}}{\text{Total volume}} \times 100$		$\dfrac{\text{Mid capacity}}{\text{Total volume}} \times 100$		$\dfrac{\text{Complementary air}}{\text{Vital capacity}} \times 100$	
	Normal	Observed	Normal	Observed	Normal	Observed	Normal	Observed
			Pulmonary emphysema					
1	78 0	45 9	22 0	54 1	38 0	70 4	79 4	64 6
2	78 0	31 7	22 0	68 3	38 0	77 0	79 4	72 6
3	78 0	23 8	22 0	76 4	38 0	87 6	79 4	45 1
4	78 0	65 2	22 0	34 8	38 0	49 1	79 4	78 2
5	78 0	64 7	22 0	35 3	38 0	57 6	79 4	65 5
6	78 0	63 2	22 0	36 8	38 0	51 4	79 4	77 0
7	78 0	70 8	22 0	29 1	38 0	38 5	79 4	88 1
8	78 0	64 3	22 0	35 7	38 0	38 7	79 4	89 3
9	78 0	28 5	22 0	71 4	38 0	80 9	79 4	66 6
			Pneumoconiosis					
10	78 0	43 6	22 0	56 4	38 0	67 3	79 4	75 0
11	78 0	63 7	22 0	36 0	38 0	50 9	79 4	76 7
12	78 0	61 5	22 0	38 4	38 0	54 4	79 4	79 7
13	78 0	69 3	22 0	30 7	38 0	43 4	79 4	84 5
14	78 0	77 1	22 0	22 9	38 0	33 6	79 4	86 1
15	78 0	51 4	22 0	48 6	38 0	56 5	79 4	84 6

respondingly increased   In severe cases the residual air occupies a much greater proportion of the total volume than the vital capacity   A profound alteration in the ability to ventilate the alveoli has accordingly taken place

In many cases especially in the severe ones, the complementary air makes up a smaller portion of the vital capacity than is the case with normal persons   This defect is a noteworthy feature in Cases 1 and 3 in which the volume of reserve air is strictly normal   It appears therefore that the distended lungs cannot be deflated to the normal level, but that a forced expiration expels nevertheless a normal quantity of air owing to the change in volume of mid capacity

When the vital capacity is composed chiefly of reserve air, or when the latter volume is not as markedly reduced as the complementary air, there is probably a further handicap to effective alveolar ventilation

Christie (2) found the volume of the reserve air was smaller after a maximum inspiration than is the case when it is measured after a normal expiration and interpreted this as an indication of diminished elasticity of
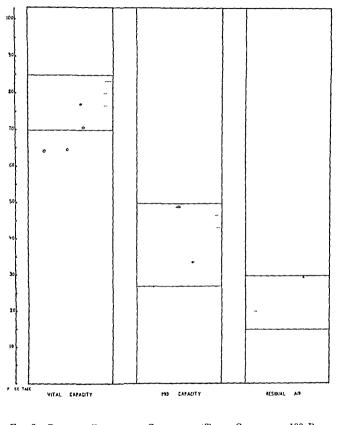
FIG 2 RELATIVE PULMONARY CAPACITIES (TOTAL CAPACITY = 100 PER
CENT) IN CASES OF PULMONARY EMPHYSEMA AND PNEUMOCONIOSIS
The shaded areas represent limits of normal variations Black dots are cases
of pulmonary emphysema circles are cases of pneumoconiosis

the lungs in pulmonary emphysema There is apparently a decrease in
the ability of individuals to deflate the lungs after they have been fully
expanded We have investigated this phenomenon in all our cases and
have found that it is not always present It occurred in two instances but
was not evident in some of the most severe and most unquestionable cases
of emphysema

Pneumoconiosis The results of our observations in this condition
differ from those obtained in pulmonary emphysema The observed total
capacity of the lungs is as a rule lower than the calculated This decrease
is proportional to the clinical and radiological evidence of the severity of
the disease The lungs of these patients contain at maximum inspiration
a smaller volume of air than would be predicted from the radiographic
measurements of the chest The diminution in total capacity is caused en-
tirely by decrease in the vital capacity The residual air is moderately
increased in almost all cases and this change indicates that there is some
degree of pulmonary emphysema associated with the fibrotic changes
This observation agrees with the frequent demonstration by pathologists
and radiologists of emphysematous areas in the lungs of these patients
The mid capacity is found to be normal in most instances The vital ca-
pacity is reduced in proportion to the total volume, and the residual air
greater but these alterations are relatively less than in cases of pulmonary
emphysema The increase in the ratio (Residual air / Total volume) is
chiefly due to diminution in the vital capacity The two components of
the vital capacity the reserve and complementary air show a normal re-
lationship to each other in pneumoconiosis

Chest expansion

Changes in the ratio (Area at maximum expiration / Area at maximum
inspiration) $\times$ 100 (measured on the doubly exposed radiograph of the
lungs) are especially interesting From previous studies (4) of normal
male subjects we concluded that a ratio higher than 72.0 may be considered
as indicating diminished expansion In all but two of the cases of pul-
monary emphysema this ratio was higher than 72 (Table 3) In these two
the diagnosis of emphysema was made on clinical grounds only In neither
of them did the pulmonary capacity show marked significant abnormality
It appears then that in undoubted cases of pulmonary emphysema there
is a conspicuous decrease in ability to expand the chest (see Figure 3)
The diaphragmatic excursion and the lateral expansion of the chest also
are as a rule decreased The angle of movement of a rib in changing from
full expiration to maximum inspiration was markedly reduced in the ma-
jority of patients In two of the severest cases movement was almost nil

In pneumoconiosis the ratio (Area at maximum expiration/ Area at
maximum inspiration) $\times$ 100 was within upper normal limits in all but
two cases In one it was markedly altered, due to reduced expansion of

TABLE 3

Chest expansion

Case number	Ratio $\dfrac{\text{Area at maximum expiration}}{\text{Area at maximum inspiration}} \times 100$	Diaphragmatic excursion *	Lateral expansion	Rib movement	Circumference expansion
		cm	cm	degrees	cm
Pulmonary emphysema					
1	90 9	0 3	2 3	20	4 0
2	86 8	3 5	2 2	7	4 5
3	84 9	2 4	0 4	3	2 0
4	77 9	5 8	2 8	14	10 0
5	70 7	5 1	1 0	11	8 0
6	75 5	4 3	2 2	22	7 0
7	77 4	3 3	1 0	12	8 0
8	66 9	4 7	0 6	10	5 0
9	82 9	4 8	0 2	2	3 0
Pneumoconiosis					
10	89 5	0 8	1 8	8	4 0
11	70 0	5 0	2 5	15	6 0
12	73 5	4 7	0 9	13	5 0
13	60 6	5 3	2 8	18	4 0
14	70 9	4 1	2 3	3	6 0
15	73 8	5 3	1 1	3	3 5

* Average of both diaphragms
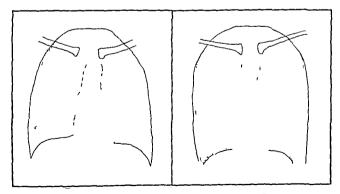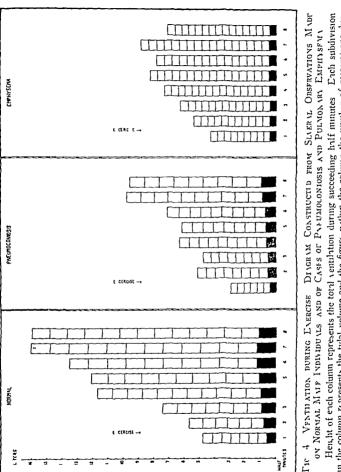
FIG 3   OUTLINE OF THE LUNG FIELDS OF MAXIMUM INSPIRATION AND EXPIRATION IN A NORMAL MAN (LEFT) AND IN A CASE OF PULMONARY EMPHYSEMA (RIGHT)

the chest   The diaphragmatic excursion the lateral expansion of the chest and the movement of the rib were usually within normal limits   Reduction in chest expansion is not characteristic of pneumoconiosis and pulmonary fibrosis, as it is of pulmonary emphysema

Relationship of dyspnea to alterations in capacity of the lungs

A striking correlation is found to exist between the observed alterations in capacity of the lungs and the tendency to dyspnea   In all those cases in which there was severe limitation in physical activity we find marked changes in the absolute and relative pulmonary capacity   A high ratio (Residual air/Total volume) is almost always accompanied by a pronounced degree of dyspnea on physical exertion   On the other hand in Cases 4, 5 7 8 and 14 in which such a ratio was either normal or but slightly increased the history of dyspnea on exertion was not conspicuous   The tendency to dyspnea in the cases of pneumoconiosis was less severe as a rule than in those with pulmonary emphysema and the changes in the relative pulmonary capacities were correspondingly not as great   In two cases in which the ratio of residual air to total volume was high dyspnea on physical activity was, however, also severe

While discussing the adaptation to physical activity it will be pertinent to present some observations on the measurement of pulmonary ventilation during exercise   The observations included graphic registration of the number of respirations tidal and minute volumes, of patient while engaged in work in a specially designed chair ergometer   These investigations are summarized graphically in Figure 4   Normally the process of adaptation to physical activity necessitates greater frequency of respiration as well as increase in the tidal volume so that the ventilation per minute is considerably increased   Peabody (6) found that efficient ventilation during exercise is closely related to the vital capacity and that when this is low the maximum possible pulmonary ventilation is reduced proportionally   In pneumoconiosis in which there is a decrease in the vital capacity patients were able to increase the number as well as the depth of the respirations during exercise yet the total ventilation was considerably less than in men   In pulmonary emphysema an abnormal response to physical exercise is evident   The vital capacity was low and there was diminished ability to expand the chest   Emphysematous individuals during physical activity tend to increase the rate rather than the depth of breathing (Fig 4)   Difficulty in expansion of the chest in emphysema is so great that when accessory muscles are brought into play voluntarily increase in the depth of breathing is accompanied by decrease in rate   In all the cases subjected to exercise tests there has been severe dyspnea on exertion and in each case the maximum ventilation attainable has been less than normal

FIG 4 VENTILATION DURING EXERCISE DIAGRAM CONSTRUCTED FROM SEVERAL OBSERVATIONS MADE ON NORMAL MALE INDIVIDUALS AND OF CASES OF PNEUMOCONIOSIS AND PULMONARY EMPHYSEMA

Height of each column represents the total ventilation during succeeding half minutes Each subdivision in the column represents the tidal volume and the figure within the column the number of respirations during that half minute

Pulmonary capacity and arterial saturation with oxygen

In seven cases the arterial blood has been examined to determine its content of carbon dioxide and the degree of saturation with oxygen   The results and the corresponding pulmonary capacities are presented in Table 4   The lowest values for saturation of arterial blood with oxygen have been found in cases in which the ratio (Residual air/Total volume) was abnormally high

TABLE 4

Relationship of arterial oxygen saturation to pulmonary capacity

Cases	Oxygen saturation arterial blood	CO content arterial blood volume	Decrease in vital capacity	Increase in residual air	Ratio Residual air / Total volume
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
(3)	72 9	49 3	−67 7	+269 1	76 2
(1)	79 6	47 6	−54 2	+90 0	54 1
(2)	80 0	46 7	−52 3	+266 0	68 3
(10)	80 6	55 2	−71 9	+29 1	56 1
(11)	81 2	46 2	−41 9	+16 9	36 3
(4)	95 6	50 2	− 8 0	+74 8	34 8
(12)	95 7	44 2	−39 5	+34 5	38 5

DISCUSSION

Marked and significant changes in the absolute and relative pulmonary capacities exist as these studies show in cases of pulmonary emphysema The results are in agreement with the investigations of Lundsgaard and Schierbeck (5) and Anthony (1), who found a normal total volume but a marked decrease in the vital capacity and a corresponding increase in the residual air   The fact that there are definite alterations in the capacity of the lungs in cases of this nature, and that these changes are closely correlated with the clinical condition and the tendency to dyspnea, is of considerable importance   The diagnosis of pulmonary emphysema is not so easy to establish clinically as it appears to be   An emphysematous appearance of the chest may be due entirely to skeletal changes without alteration in the lungs and conversely actual emphysema in the pathologic al-physiological sense of the word, may be present and may escape clinical detection because the chest is of normal size and shape   Not infrequently symptoms of respiratory failure are attributed to heart disease especially in people past middle age when pulmonary changes alone are responsible for the symptoms   In doubtful cases the measurement of the pulmonary capacity will aid in establishing the correct diagnosis   The calculated pulmonary capacity in some cases of pulmonary emphysema is not strictly to be regarded as 'normal' since we are using as a basis for prediction the size of the chest which in itself may be pathological   To be normal the capacity of the lungs in a given case must correspond to the volume of

the chest before pathological alterations occurred   We have investigated in normal men the possible correlation of thoracic volume with other bodily characteristics, and have not found any correlation by means of which we could predict normal values from other data such as height weight, or surface area   In emphysema observed total pulmonary capacities agree fairly closely with the size of the chest estimated from measurements of radiographs   The most significant alterations occur in the relative values of the subdivisions of total capacity

There are no previous observations in the literature regarding pulmonary capacity in cases of pneumoconiosis and pulmonary fibrosis   We have observed that there are significant changes in such cases, the degree of which is closely correlated with the functional efficiency of respiration and with the anatomical changes revealed in the radiological examinations From these observations it seems probable that the determination of the pulmonary capacity may be of value in the proper evaluation of the degree of respiratory disability   In Case 14 in which the evidence of fibrosis in the lungs was small the pulmonary capacity was normal   But in Cases 10 and 11 in which it was great the capacity of the lungs was much diminished   In four of five cases there was moderate increase in the volume of residual air due probably to secondary emphysema usually found in association with advanced pulmonary fibrosis

SUMMARY AND CONCLUSIONS

1   In seven of nine cases of pulmonary emphysema the total pulmonary capacity observed corresponded closely with that predicted from measurements of the chest cavity   In two it was slightly less   Increase in the volume of the residual air and a corresponding reduction in the vital capacity was observed in all cases

2   In emphysematous patients there was definite reduction in expansion of the chest the degree being closely correlated with alterations in the relative pulmonary capacities

3   In cases of pneumoconiosis (pulmonary fibrosis) the total capacity of the lungs observed was less than that predicted from measurements of the chest due to decrease in the vital capacity   The residual air was moderately increased in four of five cases   In one the changes were minimal

4   Decrease in expansion of the chest was not a significant feature of cases of pneumoconiosis (pulmonary fibrosis)

5   Cases in which the ratio (Residual air/Total volume) was abnormally high were found to exhibit low saturation of the arterial blood with oxygen indicating poor alveolar ventilation

Preliminary observations on response to exercise showed that the capacity to ventilate the lungs was limited in a severe case of emphysema and in one of pneumoconiosis compared with that in a normal man

Further observations will be necessary to establish a relation between the degree of functional disability and abnormalities in pulmonary capacity

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THE EFFECT OF DIGITALIS ON THE VENOUS PRESSURE OF NORMAL INDIVIDUALS

By DAVID A. RYFAND

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That the administration of digitalis in heart failure causes an increase in cardiac output (per minute and per beat) and a fall in venous pressure is well known (1) It is also well established that the exhibition of digitalis to normal men and dogs results in a decreased output (2 3 4 5 6 7) but data on venous pressure in normal individuals following digitalis are inconclusive

In animals Villaret Grellety Bosviel and coworkers (8) found a fall of venous pressure after intravenous injection of ouabain Kaufmann (9) Popper (10), and Dock and Tainter (5 6) found a decreased venous pressure following intravenous injection of strophanthin and digitalis Plumier (11) noted increased venous pressure after strophanthin and digitoxin Yokota (12) found a small decrease greater decrease and increase in venous pressure with intravenous injection of small medium and large doses respectively of strophanthin Capps and Matthews (13) found no change after digitalis

In man Stewart and Cohn (7) found a marked fall in venous pressure in one normal subject after oral administration of digitan using the Moritz Tabora (20) technique of venous pressure determination Simultaneously in the first subject and in two other subjects they found no constant change when a less accurate method was used (that of allowing blood to rise in a dry tube)

As Eyster (14) pointed out it is impossible to determine whether the changes produced by digitalis in output are cardiac or extra cardiac in origin unless the changes in venous pressure are known In decompensated hearts it is agreed (5 7) that the increased output is due largely to the change in ventricular tone any peripheral action of digitalis being relatively unimportant (although Wollheim (15) and others (16 17) credit the reduction in effective (systemic) blood volume with more importance in relieving congestive failure)

Opinion differs widely however regarding the origin of the digitalis effects in the normal organism Cohn and his coworkers (7 18 19) unable to demonstrate venous pressure changes hold that the decreased cardiac output is due to diminished ventricular filling caused by increased tone

of the heart muscle   Dock and Tainter (5 6) on the other hand believe that the decreased output is due to diminished ventricular filling caused by lowered venous pressure (with lessened return flow of blood to the heart), which they have shown to be the result of pooling of blood in the portal system

Grollman (1) states that   it is impossible at present to say definitely if the primary cause of the observed decrease in the cardiac output following administration of digitalis to normal individuals is due to its action on the heart or on peripheral structures   This study was designed to obtain data on the height of venous pressure before and after the administration of full doses of digitalis to normal human beings to determine whether the question of cardiac or extra-cardiac action of the drug in decreasing output could be answered

METHODS

Thirty-seven control and 82 experimental observations were made on venous pressure before and after the administration of digitalis (in single doses) in 9 trials on 8 normal subjects

Apparatus and technique   The method used was one of the direct (venepuncture) types, a simplification of the Moritz-Tabora (20) technique as suggested by Dock   The system consisted of an intravenous needle and a manometer (a straight 20 cm length of glass tubing 3 mm inside diameter), connected by means of a Kaufmann-Luer syringe   The needle and syringe were boiled before each determination, while the manometer was kept in 95 per cent alcohol

Before use, the system was connected and filled with sterile physiological saline to avoid the misleading effects of the viscosity of a blood column rising in a dry tube   The saline-filled system was suspended over the antecubital fossa of the subject and venepuncture performed in the usual aseptic manner   The plunger of the syringe was then withdrawn until the manometer was in direct communication with the vein, and fastened in place   The column of saline was allowed to fall in the manometer until equilibrium was reached when the height of the top of the column above the vein was read   Eight millimeters (found by trial) was subtracted from each reading to allow for capillarity

Each reading was made with the subject in the supine position on a thin mattress without sag so that the olecranon and the dorsum of the thorax were on the same plane   To avoid the effects of exercise (21) the subjects assumed this position 20 to 30 minutes before each determination   No readings were taken immediately after the subjects had eaten   Room temperature was approximately constant

All clothing above the waist was removed   Stasis for venepuncture was momentary only   After each reading the upper arm was compressed to insure patency of the needle and to allow the saline to rise and fall again for a confirmatory reading   Because of the number of venepunctures in each subject both arms were used, preliminary observations having shown that this proceeding introduced no error

No effort was made to approximate the level of the antecubital vein to that of the right auricle as we were more interested in following changes from hour to hour in any given subject and felt that accuracy would be sacrificed in an attempt were made to obtain absolute venous pressure (22)

Subjects There were 8 subjects 6 men and 2 women Age varied from 35 to 63 years Two series of observations were made on one woman All subjects were ambulatory patients on the neuro psychiatry ward diagnosed psychoneurosis psychasthenia involutional melancholia and schizophrenia Most of them cooperated nicely None showed any evidence of cardiovascular abnormalities in particular there were absolutely no signs or symptoms of cardiac decompensation in any subject None of these patients experienced the symptoms of pain lassitude or dyspnea noted by Stewart and Cohn (7) after digitalis administration

Digitalis The digitalis powder used was kindly supplied and standardized by Doctor A B Stockton It assayed 108 mgm to one Hatcher cat unit (predicted clinical dose = 21 to 35 mgm per kgm) and 25 mgm to one pigeon emetic dose (predicted clinical dose = 25 mgm per kgm) The dosage varied from 1 0 to 1 6 gram (19 2 to 30 0 mgm per kgm) Each subject received the powder in one dose at six o clock in the morning before breakfast

RESULTS

The initial venous pressure determinations in five of the subjects were obviously fallacious being 2 2 4 1 and 4 9 cm higher than the average of the remaining control observations in three cases and 11 0 and 3 1 cm lower in two In another instance a single reading 6 8 cm higher was found All of these erroneous values were discarded in the computations of results and do not appear in the figures or tables the net result of discarding these observations is to minimize slightly the venous pressure decrease to be pointed out below

There are 37 control observations distributed over 9 series The variabilities (100 σ/Mean) of the series are 14 1 11 7 11 4 10 1 7 3 7 1 6 4 4 2 and 3 2 per cent respectively The average variability is 8 4 per cent There are 82 observations after digitalis

An afternoon rise of venous pressure was noted in 4 of 6 series of control observations The mean afternoon rise in these 6 series was 0 4 cm of saline This is in the same direction but of much less degree than the diurnal variation noted with an indirect method by Hooker (23) No correction for this small variation was made in the computations

The control and experimental results are summarized in Table 1 (hours approximated) Table 2 and Figure 1 It is evident that digitalis caused a fall of venous pressure of greatest degree (minus 1 96 cm saline) at 24 to 32 hours after administration with a return to the control level at 72 to 96 hours

DISCUSSION

In Figure 2 (data in Table 3) is represented a comparison of our results on venous pressure (A) with those of Cohn and Stewart (4 7) on cardiac output in humans (B) and in dogs (C) All means are computed by averaging observations about their respective modes

TABLE 1

Effect of giving digitalis on venous pressure in normal individuals

Subjects*	Controls†	4	8	10	12	16	28	36	52	60	84	102	132	156	180	192
Hu ♂ wt 63, 62 kgm 12 kgm	112 118 110 105 Mean=111 Pulse=60	-15/87		+20/83			+14/87			+17/90						
Wi ♂ wt 50, 520 kgm 10 kgm	127 116 112 133 Mean=130 Pulse=101	-19/106			-03/95		-14/85									
Ol ♂ wt 88, 780 kgm 15 kgm	116 135 115 131 Mean=137 Pulse=75	-01/107	+30/107	+03/90		-02/99	-03/100				-10/88	+43/96	+20/91			-16/101
Ri ♀ wt 52, 17 kgm 10 kgm	150 115 105 120 Mean=130 Pulse=79	-26/90		+27/95		+20/99										
		-25/91		-25/83		-15/91		-10/99		+15/96						
Br ♂ wt 53, 600 kgm 13 kgm	107 125 112 117 115 Mean=133 Pulse=73	-67/110	-01/91	-01/115	-12/112	-08/104	-14/112	-55/99	-32/101	-01/101	-08/104	±00/101	-09/96	+04/101		
Sh ♂ wt 60, 517 kgm 15 kgm	91 101 105 Mean=100 Pulse=81	-10/91	-01/91	±00/99	+01/105	-01/91	-26/94	-31/91	-31/94	-27/71	-19/79					
Co ♂ wt 52, 57 kgm 16 kgm	110 121 105 123 Mean=115 Pulse=69	-10/110	-31/91	-20/90			-07/107	±00/79	-32/96	+09/101	-08/104	±00/101				
Mi ♀ wt 42, 100 kgm 12 kgm	112 88 85 101 96 Mean=96 Pulse=85	+06/111		-31/101		-13/113	-30/113			-06/103	-05/117	+05/111		-06/113	-11/99	-15/111
Mi ♀ wt 42, 100 kgm 12 kgm	92 107 81 96 Mean=92 Pulse=47	-26/57	-36/93	-22/92		-15/97	-27/110	-25/101	-07/101	-18/106	-01/110	+10/110	-02/103	-08/101	+11/99	+00/101

* Subjects are arranged in order of increasing magnitude of digitalis dosage per kilogram body weight
† Under controls are given all venous pressure observations in centimeters of saline, with their means; the means of the
control pulses (rate per minute) are also given.
‡ Figures in upper line preceded by (+) or (−) are deviations from control mean venous pressure in centimeters of
saline; lower line figures are pulse rates in percentage of control mean.

TABLE 2

Effect of giving digitalis on the venous pressure of normal individuals   Means of all observations

Hours after administration of digitalis	Range of hours after digitalis	Number of observations	Venous pressure, Deviation from control mean	Pulse rate Percentage of control mean
hours	*hours*	*number*	*cm saline*	*per cent*
9 3	4– 17	34	−1 35	97 0
31 7	28– 38	14	−1 96	98 5
57 3	52– 62	12	−1 08	95 6
88 9	76–104	10	+0 06	102 5
167 8	120–270	12	−0 28	102 1
		82		
4 8	4– 5	10	−2 26	98 3
9 2	7–10	13	−0 70	93 6
13 5	11–17	11	−1 29	99 7
		34		

In the lower part of the table, the observations in the first 17 hours are further separated   The comparatively high venous pressure in the 7–10 hour range is due to the slow pulse (93 6 per cent)   See discussion in text.

Direction of changes   Digitalis, in normal individuals, causes a *decrease* in both cardiac output and venous pressure   It is axiomatic that a cardiac cause for diminished output will result in an increased venous pres-
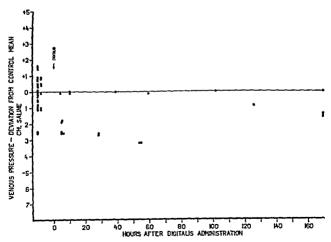
Fig 1   Scatter Diagram Showing All Observations

sure, and that an extra-cardiac production of lowered venous pressure will bring about a decreased cardiac output

Duration of changes The time relations of venous pressure changes to cardiac output changes are similar The lowest mean venous pressure

FIG 2   THE EFFECT OF DIGITALIS ON VENOUS PRESSURE AND CARDIAC
OUTPUT IN NORMAL INDIVIDUALS

A Venous pressure   *B* Output in human beings (7)   *C* Output in dogs
(4)

occurred at 31 7 hours, while the minute and stroke volume in man fell to their lowest values at 24 hours (after oral administration of digitalis) The 8 hour difference is probably due to the paucity of observations and to the necessity of averaging observations about their modes   In dogs, be-

TABLE 3
The effects of digitalis administration on venous pressure and on cardiac output A comparison

Hours after digitalis administration	Number of observations	Venous pressure Deviation from control mean	Stroke volume Percentage of control	Minute volume Percentage of control	Pulse rate Percentage of control
hours	*number*	*cm saline*	*per cent*	*per cent*	*per cent*
		A Venous pressure in the normal human being			
9 3	34	−1 35			97 0
31 7	14	−1 96			98 5
57 3	12	−1 08			95 6
88 9	10	+0 06			102 5
		B Cardiac output in the normal human being (7)			
2	2		105 5	100 5	95 5
6	10		93 4	86 4	92 5
13	8		89 3	82 1	92 2
24	7		85 4	80 1	95 4
48	6		95 6	95 2	99 1
72	4		99 0	96 2	101 5
		C Cardiac output in the normal dog (4)			
2 5	11		72 9	56 5	80 9
25 5	12		62 2	71 2	112 5
48	3		78 4	83 0	103 7
73	4		100 5	103 8	104 5

cause of the intravenous administration of the drug, time relationships are not strictly comparable, even here, however, the stroke volume is lowest at 25 5 hours

Venous pressure returned to the control level between 72 and 96 hours after digitalis   Cardiac output in man reached normal values in 72 hours (stroke volume 99 0 and minute volume 96 2 per cent of their respective controls)   Cardiac output in dogs was slightly above the control in 73 hours   (The apparent 16 day duration of depressed output in Stewart and Cohn s (7) Subject 1 is undoubtedly due to a high control reading   for the values at 4, 5, 7, and 16 days after digitalis are remarkably constant—87, 89, 88, and 82 per cent of the control minute volume respectively)

The effect of digitalis on the A–V conduction time and T wave of the electrocardiogram disappears in 5 to 22 days (24 25)   We made no electrocardiographic studies, but with the doses used the calculated duration of conduction time and T wave effects in our series would be 8 to 14 days (26)

The duration of digitalis action on both cardiac output and venous pressure is more fleeting, then, than on the central T wave and A–V con-

sure, and that an extra-cardiac production of lowered venous pressure will bring about a decreased cardiac output

Duration of changes   The time relations of venous pressure changes to cardiac output changes are similar   The lowest mean venous pressure

HOURS AFTER DIGITALIS ADMINISTRATION

FIG 2   THE EFFECT OF DIGITALIS ON VENOUS PRESSURE AND CARDIAC OUTPUT IN NORMAL INDIVIDUALS

A Venous pressure   *B* Output in human beings *(7)*   *C* Output in dogs *(4)*

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be regarded as constant, and the effects on diastolic size, as measures of the effect of digitalis on tone " Obviously, heart work is a function of the output per beat and is not, as Cohn and Steele suggest, measured by the oxygen consumption of the entire organism   Starling and Visscher (29) have demonstrated that diastolic volume varies with heart work, so that the decreased size of the heart observed by Cohn and Stewart (4, 7) should occur as a result of the decreased output   In expelling equal quantities of blood, a small heart must make greater excursions than does a larger heart This fact amply explains the observations of Cohn and Stewart (4) without the necessity of invoking changes of tone   The effect is great enough to exist despite the decreased volume which the small heart ejects after digitalis

Cohn and Steele (18) further state " the decrease in volume output which has been noticed was thought to result from the tonic effect of digitalis upon the heart (4), in consequence of which its size diminished so much, that a large or even a usual volume of blood could not be received and, therefore, could not be ejected ' If this were the case, venous pressure should rise as it does in cardiac tamponade (27) , we have demonstrated on the contrary, that venous pressure actually falls   Moreover, Wiggers and Stimson (30) showed by intraventricular pressure curves that the heart under digitalis offers no more resistance to inflowing blood than does the normal undigitalized heart

Finally, Tainter (31) was able to reproduce the typical digitalis effects of decreased output and lowered venous pressure in cats with the Gibbs artificial heart apparatus in which preparation the heart is replaced by a mechanical pump and in which, therefore there could be absolutely no question of changes in " heart tone "

Relation of venous pressure to pulse rate   If digitalis bradycardia were due to the Bainbridge reflex, following decreased auricular pressure, we should expect the greatest slowing of pulse to occur in those subjects with the largest fall of venous pressure   However, Figure 3 (data in Tables 2 and 4) shows that the opposite is the case (the increased venous pressure in Figure 3 C is due to errors of selection because of the small number of observations)

The fact that the pulse rate mean chanced not to fall below 95 per cent of the control in our series in no way invalidates the significance of the venous pressure decrease, for in the dogs of Cohn and Stewart (4), the lowest outputs were recorded with a mean pulse rate of 112 5 per cent of the control   Burwell, Neighbors, and Regen (3) also noted that output returned toward normal when their subjects experienced nausea or had more rapid pulses   In one patient with acute hepatitis (not included in this series) in whom digitalis produced partial heart block (pulse rate 41 to 47) and intense nausea and vomiting of four days duration the venous pressure fell only 1 4 and 0 8 cm at 5 and 11 hours respectively   The ef-

duction time changes   It is more prolonged than the rise of arterial pressure in dogs

Degree of changes   Dock and Tainter (5) found in their dogs F4 and F5 that falls in venous pressure of 3 5 and 6 5 cm of water corresponded to minute output reductions of 41 and 55 per cent of controls, respectively On the average, 1 0 cm of venous pressure was equivalent to 10 1 per cent of minute volume   Beck and Isaac (27) found, in experimental cardiac tamponade studies, that a 3 3 cm rise in venous pressure was associated with a 36 per cent reduction in cardiac output, and a 10 0 cm rise with a 64 per cent reduction   Thus, 1 0 cm of venous pressure was equivalent to 8 6 per cent of minute volume   Wiggers (28) presents a figure showing increasing systolic discharge in parallel with experimentally increased venous pressure (heart rate constant)   Measurements taken from this figure permit the calculation that 1 0 cm of venous pressure is equivalent to 10 87 per cent of stroke volume

Figure 2, in which the venous pressures of one group of subjects are compared with the cardiac outputs of a similarly studied group, shows that at the time of the maximal digitalis effect in man the venous pressure had fallen 1 96 cm and the minute and stroke volumes 19 9 and 14 6 per cent of their respective controls   that is, a 1 0 cm venous pressure change corresponded to a 10 15 and a 7 45 per cent change in minute and stroke volumes

From a comparison of our results with those of Cohn and Stewart (4, 7), it is evident that venous pressure and cardiac output changes in normal individuals after digitalis correlate remarkably well in direction, time, and degree   The observations on direction can be explained only by a peripheral mechanism of digitalis action, those on time support such a mechanism, for the change in venous pressure and cardiac output (like that in arterial pressure in dogs) is less prolonged than the effect on the T wave and conduction time, the degree of the venous pressure change is sufficient to explain the observed fall of cardiac output   The correlation which Stewart and Cohn (7) make between output and cardiac area is not so well marked as that between output and venous pressure, and neither supports nor controverts either the cardiac or extra-cardiac hypothesis of digitalis action

The question of cardiac tone   According to Cohn and Steele (18), digitalis decreases cardiac output in normal cardiovascular systems by an increase in cardiac tone   the only evidence presented that the tone is increased is that of Cohn and Stewart (4)   The latter observers found, radiographically, that the hearts of normal dogs became smaller and exhibited greater ventricular excursions after digitalis

Cohn and Steele (18) state " since, in the experiments of Cohn and Stewart (4), the measurements were made during periods v hen the dogs were in so-called basal states, the work which their hearts performed may

be regarded as constant, and the effects on diastolic size, as measures of the effect of digitalis on tone " Obviously, heart work is a function of the output per beat and is not, as Cohn and Steele suggest, measured by the oxygen consumption of the entire organism Starling and Visscher (29) have demonstrated that diastolic volume varies with heart work, so that the decreased size of the heart observed by Cohn and Stewart (4, 7) should occur as a result of the decreased output In expelling equal quantities of blood, a small heart must make greater excursions than does a larger heart This fact amply explains the observations of Cohn and Stewart (4) without the necessity of invoking changes of tone The effect is great enough to exist despite the decreased volume which the small heart ejects after digitalis

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fect of digitalis in reducing venous pressure is powerful enough, then, to overcome the counteracting effect of digitalis bradycardia (which, unopposed, would increase auricular pressure)
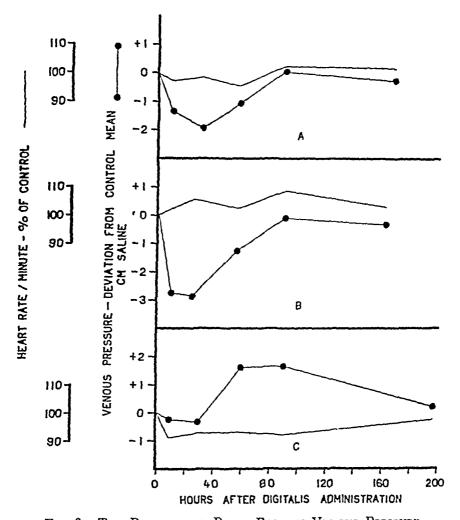
FIG 3   THE RELATION OF PULSE RATE TO VENOUS PRESSURE
   A Means of all observations   *B* Means of those with least slowing of pulse   *C* Means of those with greatest slowing of pulse   (In *C* the apparent rise of venous pressure is due to the small number of observations between 40 and 120 hours after giving digitalis)

   The production of decreased venous pressure   The hypothesis advanced by Dock and Tainter (5, 6, 31) to account for the lowered venous pressure and cardiac output after digitalis is that this drug constricts the hepatic veins, resulting in diminished return flow through the inferior vena cava   References to earlier workers on this " sluice mechanism " are given in their papers, along with their corroboratory experimental data   The

TABLE 4
Relation of venous pressure to pulse rate

Hours after digitalis administration	Number of observations	Venous pressure Deviation from control mean	Pulse rate Percentage of control
hours	*number*	*cm. saline*	*per cent*
A Means of all subjects			
9 3	34	−1 35	97 0
31 7	14	−1 96	98 5
57 3	12	−1 08	95 6
88 9	10	+0 06	102 5
167 8	12	−0 28	102 1
B Means of subjects with least slowing of pulse in the first 36 hours			
9 4	15	−2 75	102 4
25 6	7	−2 88	105 8
56 3	7	−1 24	102 3
88 9	7	−0 11	108 7
161 8	10	−0 38	103 0
C Means of subjects with greatest slowing of pulse in the first 36 hours			
9 0	14	−0 24	91 0
30 7	4	−0 33	92 7
60 5	2	+1 60	93 0
89 2	2	+1 65	92 0
197 6	2	+0 20	97 5

hypothesis is confirmed by the results of other workers (15, 16 17)   Cohn and Steele (18) state that " in order to be effective in shutting off from the volume of blood returning to the right auricle as much as a quarter, the lumina of the two vessels " [right and left hepatic veins] " must be reduced to zero—must be completely obliterated "   However, Blalock and Bradburn (32) produced a 75 per cent reduction in cardiac output by intestinal trauma and associated altered circulatory state in the portal area, Elman and Cole (33) found that approximately 60 per cent of the total blood volume accumulates in the splanchnic area within a short time after portal vein ligation, Enderlen and coworkers (34) found that death occurs from reduced arterial pressure and cardiac output in 10 to 60 minutes after portal vein occlusion   With such dramatic results following the prevention of portal outflow, it is easily possible that digitalis should lower cardiac output only 20 per cent and venous pressure 2 cm by constricting hepatic vein radicles

The anatomic evidence for the " sluice mechanism ' has been investigated in cats dogs and human beings by Elias and Feller (36) Popper

(37), and Bauer, Dale, Poulsson, and Richards (35), the subject is reviewed by the latter In brief, the findings suggest that in the dog the mechanism is brought about by the anatomic construction at the caval openings of the hepatic veins, in the cat and in man the hepatic vein radicles probably play a more important role Whether the greater sensitivity of the dog to digitalis action on cardiac output (Fig 2) may be attributed to the anatomic findings is not known

Clinical applications The reduction in heart size noted by Stewart and Cohn (7) is undoubtedly due to the digitalis effects studied above, such action, if sustained, would retard cardiac hypertrophy In a recent paper, Christian (38) proposes continuous complete digitalization of patients with enlarged hearts (and of those in whom cardiac enlargement may be expected) while compensated, under the clinical impression that enlargement and decompensation are retarded Because of the importance of this subject, the impression should be confirmed or disproved by experiments designed to determine how long the digitalis effect on heart size, venous pressure, and cardiac output can be sustained in animals, normal human beings, and those compensated patients whom Christian suggests digitalizing to retard heart failure

CONCLUSIONS

1 Digitalis causes, in normal human beings and dogs, a decreased cardiac output and a decreased venous pressure The greatest effect occurs at about 24 to 32 hours after administration of the drug, with a return to normal levels in 72 to 96 hours

2 The observed changes support the hypothesis that digitalis owes its action to a peripheral effect, probably on the hepatic vein radicles, in reducing the return flow of blood to the heart The hypothesis that the digitalis action follows changes in cardiac tone is negatived by all available data

3 Digitalis bradycardia is not due to the fall of venous pressure On the contrary, the slowing of the heart, by causing the normal increase in venous pressure, partially conceals the effect of digitalis in reducing the return flow of blood to the heart

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A COMPARISON OF THE CREATININE AND UREA CLEARANCE TESTS OF KIDNEY FUNCTION

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The urea clearance test developed by Möller, McIntosh and Van Slyke (1) has one disadvantage, namely, that maximum and standard clearances are only comparable by reference to the average normal value for each or by multiplying the standard clearance by a constant   In addition the standard clearance, involving the square root of the urine volume is difficult to interpret   The creatinine clearance test, developed by Rehberg (2), is not subject to these objections, since the excretion of creatinine is independent of urine volume   It therefore seemed worth while to compare the creatinine and urea clearances as a test of renal function, to determine if there was sufficient practical advantage in the creatinine test to compensate for the added technical difficulties

Rehberg presented evidence that led him to believe that the creatinine clearance represented the volume of glomerular filtrate and on this assumption calculated the quantities of urea and chloride reabsorbed   Rehberg s conception has been accepted by Wyschegorodzewa (3), Bergwall (4), and others (5 6), questioned by Cope (7) and Ekehorn (8), and denied by Shannon, Jolliffe and Smith (9)   The evidence at present available does not, in our opinion justify unreservedly accepting the creatinine clearance as equal to the volume of glomerular filtrate, and while this lessens the value of the test as a tool in the study of renal physiology it need not detract from its usefulness as a practical test of kidney function

METHODS

Hospital patients were kept in bed for the period of the test   Dispensary patients and normal subjects were allowed to sit in a chair or engage in light laboratory work   Some tests were made after fourteen hours fast the majority after a light breakfast which MacKay (10) found had no effect on the urea clearance   Three to five grams of creatinine were given by mouth an hour to one and one-half hours before the beginning of the test   At the beginning of the test the bladder was emptied as completely as possible and a sample of venous blood obtained   Approximately an hour later, but timed to the nearest minute, the bladder

was again emptied as completely as possible and a second sample of blood obtained   If there was any doubt whether the subject could empty the bladder, he was catheterized   All blood analyses were made on serum

Urea nitrogen in urine and serum was estimated by the gasometric method of Van Slyke (11), in a few instances by Van Slyke and Cullen's (12) method   All analyses were made in duplicate   Creatinine in urine and serum was estimated by Rehberg's (13) modification of Folin's method using a colorimeter with Burker optical system

The ingestion of three to five grams of creatinine increases the plasma concentration to 5 to 10 mgm per cent   This decreases the effect of substances other than creatinine in plasma which give the Jaffe reaction as well as making the estimation more certain   Since Behre and Benedict (14) and Gaebler (15) have doubted the existence of creatinine in normal blood, and since Gaebler could recover only a relatively small fraction of the creatinine added to blood it was felt that the creatinine analyses had to be examined before any reliance could be placed on the calculation of creatinine clearance by the formula $UV/B = C$ By the method used in these experiments the average recovery of creatinine added to serum in amounts equivalent to 0 5 to 15 0 mgm per 100 cc was 93 5 per cent in 15 experiments, the extremes being 80 and 124 per cent   Table I shows

TABLE I

Comparison of creatinine clearances calculated from total chromogenic substances and from ingested creatinine only

Name	Before Ingestion		After Ingestion		Creatinine clearance †	
	Serum creatinine	Creatinine excreted	Average serum creatinine	Creatinine excreted	A	B
	mgm per cent	*mgm per minute*	*mgm per cent*	*mgm per minute*	*A*	*B*
J M H	1 27*	1 42*	9 63	12 72	132	134
			8 31	10 30	124	126
			12 22	12 50	102	101
White	0 95	1 37	6 16	10 05	163	166
Wilson	1 08	1 59	8 28	8 08	106	100
Letcher	1 01	89	9 20	11 05	120	121

* Average of 4 determinations
† A—clearance calculated from total chromogenic substance in serum and urine
   B—clearance calculated from ingested creatinine only, subtracting value of chromogenic substances normally present in serum and average rate of excretion of creatinine before ingestion

that there is no difference in the clearance calculated on total chromogenic substances in serum and urine after ingestion of creatinine and in the clearance calculated on ingested creatinine only   Gaebler and Keltch (16) have shown that all the chromogenic material adsorbed on Lloyd's reagent

and released again is indistinguishable from creatinine   Therefore, a clearance calculated on the material released from Lloyd's reagent should be higher than that calculated on total chromogenic substances when no creatinine has been fed, since other substances account for a considerable fraction of the value obtained for " creatinine " in normal serum   After ingestion of creatinine, the clearances calculated before and after the use of Lloyd's reagent should show much less difference   Table II shows that

TABLE II

Comparison of creatinine clearances calculated from total chromogenic substances and from material released from Lloyd s reagent with and without ingestion of creatinine

Experimental number and condition		Urine volume	Total creatinine			Released from Lloyd s		
			Serum	Urine	Clearance	Serum	Urine	Clearance
		cc per minute	*mgm per cent*	*mgm per cent*	*cc per minute*	*mgm per cent*	*mgm per cent*	*cc per minute*
1	No	6 84	1 01	131	89	84	136	111
2	creatinine	7 07	1 30	20	109	96	18	133
3	ingested	7 75	1 32	18	106	87	14	125
4	Creatinine	7 72	9 63	165	132	9 03	159	136
5	ingested	1 95	12 22	642	102	10 99	600	106
6		9 35	6 16	108	164	6 02	101	157

this is the case   These experiments led us to believe that with serum concentrations of creatinine above 5 mgm per cent the creatinine clearance could be calculated on total chromogenic substance present in serum and urine without significant error

In order to estimate the range of creatinine clearance in normal individuals under conditions of hospital and office practice 59 clearances on 59 apparently healthy individuals were tabulated   In 45 of these, only a single test was made   In 14, from 2 to 21 clearances were determined For these the first clearance determined was tabulated   The range in these 59 observations was from 87 to 232 cc per minute, the mean 144 cc per minute with a standard deviation of 36 cc. When repeated tests are made of the same individual, there is a similar wide dispersion, but the limits for all clearances which we have obtained from normal individuals are only slightly wider   Nor apparently does any normal individual tend to have a constantly high or low clearance

For this reason, it seemed proper to include all our observations (130) of normal individuals where no recognized unusual factor, such as administration of a drug or exercise, was present to show the normal chance distribution of clearances   If this be done, the range is from 70 to 238 cc. per minute, the mean 148 cc per minute with a standard deviation of 34 cc A frequency polygram of these observations shows a decided skewness, but not sufficient to make unreasonable the belief that the chances of a single

was again emptied as completely as possible and a second sample of blood obtained   If there was any doubt whether the subject could empty the bladder, he was catheterized   All blood analyses were made on serum

Urea nitrogen in urine and serum was estimated by the gasometric method of Van Slyke (11), in a few instances by Van Slyke and Cullen's (12) method   All analyses were made in duplicate   Creatinine in urine and serum was estimated by Rehberg's (13) modification of Folin's method, using a colorimeter with Burker optical system

The ingestion of three to five grams of creatinine increases the plasma concentration to 5 to 10 mgm per cent   This decreases the effect of substances other than creatinine in plasma which give the Jaffe reaction, as well as making the estimation more certain   Since Behre and Benedict (14) and Gaebler (15) have doubted the existence of creatinine in normal blood, and since Gaebler could recover only a relatively small fraction of the creatinine added to blood, it was felt that the creatinine analyses had to be examined before any reliance could be placed on the calculation of creatinine clearance by the formula $UV/B = C$ By the method used in these experiments the average recovery of creatinine added to serum in amounts equivalent to 0 5 to 15 0 mgm per 100 cc was 93 5 per cent in 15 experiments, the extremes being 80 and 124 per cent   Table I shows

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Comparison of creatinine clearances calculated from total chromogenic substances and from ingested creatinine only

Name	Before Ingestion		After Ingestion		Creatinine clearance †	
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			8 31	10 30	124	126
			12 22	12 50	102	101
White	0 95	1 37	6 16	10 05	163	166
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* Average of 4 determinations

† *A*—clearance calculated from total chromogenic substance in serum and urine

B—clearance calculated from ingested creatinine only, subtracting value of chromogenic substances normally present in serum and average rate of excretion of creatinine before ingestion

that there is no difference in the clearance calculated on total chromogenic substances in serum and urine after ingestion of creatinine and in the clearance calculated on ingested creatinine only   Gaebler and Keltch (16) have shown that all the chromogenic material adsorbed on Lloyd's reagent

This is a lower normal limit than Holten and Rehberg (19) use   They found the clearance always above 100 cc per minute in normal individuals when tested between 10 and 11 a m   But in 89 clearances reported by Rehberg of himself where experimental conditions were more varied regarding posture and fluid intake, the clearance was 9 times between 80 and 100 cc per minute, and twice between 60 and 80 cc per minute, so that a patient cannot be said to have diminished renal function on the basis of a single creatinine clearance test between 60 and 100 cc per minute

The maximum urea clearance in 56 observations of 25 normal individuals ranged from 38 to 112 cc. per minute, with an average of 74 68 cc per minute and a standard deviation of 17 57 cc , identical with the average of 75 cc per minute found by Möller McIntosh and Van Slyke The range in our subjects is somewhat greater than the extremes of 52 2 and 103 8 cc per minute which they found   The mean standard urea clearance in 39 observations of 26 persons was 51 cc per minute with a standard deviation of 10 11 cc   The range was from 30 to 67 cc per minute   This is the same range found by Möller, McIntosh and Van Slyke (28 3 to 69 3 cc per minute) while the mean is but slightly lower Thus in the case of normal individuals creatinine and urea clearances have about the same degree of dispersion   The creatinine clearance is always numerically greater than the maximum urea clearance, although when the standard urea clearance is calculated using the square root of urine volume, the numerical value may be greater for urea than for creatinine clearance In estimating reduction of kidney function we have used 148 cc per minute as the average normal creatinine clearance and 75 cc and 54 cc per minute as the average normal maximum and standard urea clearances

A comparison of creatinine and urea clearances has been made 116 times on 93 patients with Bright's disease and certain other conditions (Tables III, IV and V)   We have followed Addis's classification (20) of Bright's disease but have used the term acute instead of initial hemorrhagic Bright's disease   Patients with degenerative Bright's disease, of whom relatively few were examined, have been grouped with the miscellaneous cases in Table V   We have not had the opportunity of examining any patient in whom the diagnosis of " cryptic " degenerative Bright's disease (pure lipoid nephrosis) seemed proper   Cases 107–624 and 151–532 presented a typical picture at the time of some examinations, but had had hematuria some time previously   Both clearance tests frequently show marked reduction in kidney function before there is any elevation of blood urea nitrogen, decrease in two hour phenolsulphonphthalein output, or fixation of specific gravity   This was shown for the urea clearance by Van Slyke and his associates (21) and for creatinine by Holten and Rehberg (19)   We have not found the decrease more constant or more marked in one test than the other   Just as in normal persons, the creatinine clearance is always numerically greater than the maximum urea clearance, the

clearance of a normal adult falling between 80 and 216 are about 24 1 (Fig 1)

McIntosh, Möller and Van Slyke (17) found that more constant normal values for urea clearance were obtained if a correction was made for surface area   This seems reasonable for Taylor, Drury and Addis (18) were able to show that kidney weights in rabbits varied in proportion to surface area rather than to body weight   To avoid an error greater than ± 5 per cent in using the formula $UV/B = C$, body size can be neglected only in adults between 164 and 176 cm in height   We have corrected the creatinine clearance values by the factor $(175/A)$ where A is surface area
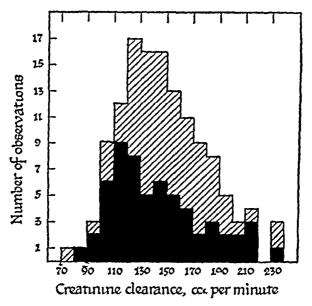
FIG 1   FREQUENCY POLYGRAM OF 130 CREATININE CLEARANCE VALUES ON 59 NORMAL ADULTS

The solid area includes only the first observation made on each person

in square meters   This amounted to more than 10 per cent in 5 instances The average of the corrected clearances is 145 ± 34 cc per minute, and the dispersion practically the same as for the uncorrected values   Since other unknown factors produce a much greater variation in clearances done on the same individual at different times than the amount of the correction for surface area, we feel that this correction is unnecessary in the case of adults except possibly for those of extreme heights   We have not applied the correction in the patients studied

From our experience with normal subjects and patients suffering from various diseases, we have come to believe that creatinine clearances below 60 cc per minute (41 per cent of average normal) are definitely abnormal, and those between 60 and 80 cc per minute (41 to 54 per cent average normal) doubtful but significant if a repeated test is in the same range

This is a lower normal limit than Holten and Rehberg (19) use   They found the clearance always above 100 cc per minute in normal individuals when tested between 10 and 11 a m   But in 89 clearances reported by Rehberg of himself where experimental conditions were more varied regarding posture and fluid intake, the clearance was 9 times between 80 and 100 cc per minute, and twice between 60 and 80 cc per minute, so that a patient cannot be said to have diminished renal function on the basis of a single creatinine clearance test between 60 and 100 cc per minute

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TABLE III

Creatinine and urea clearances in patients with hemorrhagic Bright's disease

Hospital number	Sex	Age	Date	Stage of disease	Blood pressure	Phenol-sulphon-phthalein	Blood urea nitrogen	Urea clearance	Per cent of normal	Creatinine clearance	Per cent of normal	Date of death
		years			mm Hg	per cent in 2 hours	mgm per cent	cc per minute		cc per minute		
116-625	F	14	May 17, 1932	Acute	110/70	80	16.5	32.8	44	55	37	
116-212	M	33	January 28, 1932	Acute	110/100		33.6	51.0	68	94	61	
136-555	M	20	January 26, 1933	Acute	135/80	55	10.0	32.8*	61	83	56	
118-411	M	55	October 26, 1932	Acute	110/80	30	25.3	33.3	44	80	54	
150-822	F	27	February 23, 1933	Acute	115/80		9.8	92.5	123	166	112	
139-831	M	12	June 25, 1931	Acute	190/120	20	34.9	12.7*	24	39	26	
			August 27, 1931	Latent	110/90	70	14.0	21.9*	41	69	47	
			December 22, 1932	Latent	120/80		18.0	36.9	49	88	59	
			January 5, 1933	Latent			9.7	31.2	16	73	19	
			January 19, 1933	Latent			21.7	43.2	58	95	64	
			January 23, 1933	Latent			31.4	22.3*	41	66	45	
			January 30, 1933	Latent			9.2	27.8	37	73	49	
150-571	M	38	January 1, 1933	Chronic active	180/100	68	18.2	21.4	29	11	28	
116-216	M	52	April 26, 1932	Chronic active	130/80	30	26.6	16.6*	31	86	58	
107-621	M	15	January 12, 1932	Chronic active	115/70		8.5	62.2	83	206	139	
			April 25, 1932	Chronic active	115/15	67	79.8	9.1*	17	30	20	
119-975	F	38	November 21, 1932	Chronic active	110/100	18	24.2	29.1*	51	89	60	
139-722	M	45	October 8, 1931	Chronic active	115/80	20	28.8	11.6	19	28	19	
119-016	F	21	November 22, 1932	Chronic active	160/110	50	17.6	33.4	15	77	52	
150-097	M	32	November 29, 1932	Chronic active		65	21.1	18.8*	35	62	12	
			December 9, 1932	Chronic active	105/70	70	11.2	50.0*	93	95	64	

TABLE III—(Continued)

Hospital number	Sex	Age	Date	Stage of disease	Blood pressure	Phenol sulphon phthalein	Blood urea nitrogen	Urea clearance	Per cent of normal	Creatinine clearance	Per cent of normal	Date of death
		years			mm Hg	per cent in 2 hours	mgm per cent	cc per minute		cc per minute		
151-107	F	35	February 10 1933	Chronic active	120/70	58	16 0	44 0*	82	79	53	
118-347	M	54	October 2 1931	Chronic active	130/80	60	7 5	67 0	89	88	60	
142-123	M	51	November 2 1931	Chronic active	140/100	50	11 3	34 9	47	47	32	
144-018	F	28	September 28, 1931	Chronic active	155/85	60	19 0	24 4	33	70	47	
142-730	M	34	December 8, 1931	Chronic active	165/90	65	14 9	24 6*	46	53	36	
			October 2, 1931	Chronic active	185/110	40	14 0	18 2*	34	47	32	
			October 10 1932	Chronic active	205/135	10	30 3	13 8*	26	29	20	
86-642	F	31	January 4 1933	Terminal	190/130	10	28 6	14 9	20	20	14	March 1, 1933
			October 28 1932	Chronic active	110/80	25	24 0	11 9	16	28	19	
			January 25 1933	Chronic active			24 7	9 3	12	26	18	
145-429	M	43	March 1 1932	Latent	135/80	70	14 0	101 0	135	149	101	
0-343-184	F	59	April 6, 1933	Latent	160/100		8 9	67 0	81	126	85	
134-772	M	52	July 20 1931	Terminal	160/110		12 4	21 5*	40	46	31	November 28 1932
89-697	M	22	February 4, 1932	Terminal	180/110	5	111 0	3 9*	7	6	5	May 6, 1932
145-811	M	49	March 28 1932	Terminal	140/110	5	188 0	0 6*	1	0 5	0 34	April 1, 1932
117-565	F	20	October 19 1931	Terminal	150/110	10	52 4	3 8*	7	8 0	50	February 21, 1933
151-508	F	27	March 3 1933	Terminal	260/150	10	70 0	5 5*	10	12 0	8	March 4, 1933
147-812	F	49	July 26 1932	Terminal	110/60	5	119 0	0 9*	2	0 4	0 3	July 28 1932

* Standard clearances

TABLE III

Creatinine and urea clearances in patients with hemorrhagic Bright's disease

Hospital number	Sex	Age	Date	Stage of disease	Blood pressure	Phenol-sulphon phthalein	Blood urea nitrogen	Urea clearance	Per cent of normal	Creatinine clearance	Per cent of normal	Date of death
		years			mm Hg	per cent in 2 hours	mgm per cent	cc per minute		cc per minute		
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118–311	M	55	October 26, 1932	Acute	110/80	30	25.3	33.3	41	80	51	
150–822	F	27	February 23, 1933	Acute	115/80		9.8	92.5	123	166	112	
139–831	M	12	June 25, 1931	Acute	190/120	20	34.9	12.7*	21	39	26	
			August 27, 1931	Latent	110/90	70	14.0	21.9♦	41	69	47	
			December 22, 1932	Latent	120/80		18.0	36.9	49	88	59	
			January 5, 1933	Latent			9.7	34.2	16	73	19	
			January 19, 1933	Latent			21.7	43.2	58	95	61	
			January 23, 1933	Latent			34.4	22.3*	41	66	15	
			January 30, 1933	Latent			9.2	27.8	37	73	49	
150–571	M	38	January 4, 1933	Chronic active	180/100	68	18.2	21.4	29	11	28	
116–216	M	52	April 26, 1932	Chronic active	130/80	30	26.6	16.6*	31	86	58	
107–621	M	15	January 12, 1932	Chronic active	115/70		8.5	62.2	83	206	139	
119–975	F	38	April 25, 1932	Chronic active	115/45	67	79.8	9.1*	17	30	20	
130–722	M	15	November 21, 1932	Chronic active	140/100	48	24.2	29.1*	54	89	60	
119–916	F	21	October 8, 1931	Chronic active	115/80	20	28.8	11.6	19	28	19	
150–097	M	32	November 22, 1932	Chronic active	160/110	50	17.6	33.4	45	77	52	
			November 29, 1932	Chronic active	105/70	65	21.1	18.8*	35	62	12	
			December 9, 1932	Chronic active		70	14.2	50.0*	93	95	64	

TABLE III—(Continued)

Hospital number	Sex	Age	Date	Stage of disease	Blood pressure	Phenol sulphon phthalein	Blood urea nitrogen	Urea clearance	Per cent of normal	Creatinine clearance	Per cent of normal	Date of death
		years			mm Hg	per cent in 2 hours	mgm per cent	cc per minute		cc per minute		
151–107	F	35	February 10 1933	Chronic active	120/70	58	16 0	44 0*	82	79	53	
118–347	M	54	October 2, 1931	Chronic active	130/80	60	7 5	67 0	89	88	60	
			November 2 1931	Chronic active	140/100	50	11 3	34 9	47	47	32	
142–123	M	51	September 28 1931	Chronic active	155/85	60	19 0	24 4	33	70	47	
144–018	F	28	December 8 1931	Chronic active	165/90	65	14 0	24 6*	46	53	36	
142–730	M	34	October 2, 1931	Chronic active	185/110	40	14 0	18 2*	34	47	32	
			October 10 1932	Chronic active	205/135	10	30 3	13 8*	26	29	20	
			January 4 1933	Terminal	190/130	10	28 6	14 9	20	20	14	March 1 1933
86–642	F	31	October 28 1932	Chronic active	110/80	25	24 0	11 9	16	23	19	
			January 25 1933	Chronic active			24 7	9 3	12	26	18	
145–429	M	43	March 1 1932	Latent	135/80		14 0	101 0	135	149	101	
0–343–184	F	59	April 6 1933	Latent	160/100	70	8 9	67 0	81	126	85	
134–772	M	52	July 20, 1931	Terminal	180/110		12 4	21 5*	40	46	31	November 28, 1932
89–697	F	22	February 4 1932	Terminal	140/70	5	111 0	3 9*	7	6	5	May 6, 1932
145–811	M	49	March 28 1932	Terminal	150/110	5	188 0	0 6*	1	0 5	0 34	April 1 1932
117–565	F	20	October 19 1931	Terminal	150/110	10	52 4	3 8*	7	8 0	5 0	February 21, 1933
151–508	F	27	March 3 1933	Terminal	260/150	10	70 0	5 5*	10	12 0	8	March 4, 1933
147–812	F	49	July 26 1932	Terminal	110/60	5	119 0	0 9*	2	0 4	0 3	July 28 1932

* Standard clearances

TABLE IV

Creatinine and urea clearances in patients with arteriosclerotic Bright's disease

Hospital number	Sex	Age	Date	Blood pressure	Phenol-sulphon-phthalein	Blood urea nitrogen	Urea clearance	Per cent of normal	Creatinine clearance	Per cent of normal	Date of death
		years		mm Hg	per cent in 2 hours	mgm per cent	cc per minute		cc per minute		
0-318-091	F	11	April 6, 1933	220/110		13.5	46.8	62	116	78	
105-700	M	11	January 19, 1933	260/160	30	13.2	41.7*	77	111	75	
126-997	M	51	October 6, 1931	150/110	50	11.2	31.8	46	98	66	
115-997	M	29	April 7, 1932	200/130	52	16.9	31.8*	59	98	66	June 28, 1932
			July 29, 1932	230/170	25	31.9	26.8*	50	47	32	August 27, 1932
119-734	F	60	November 23, 1932	110/80		15.4	44.8*	83	91	62	
0-330-611	M	62	January 17, 1933	200/100	55	20.0	37.8*	70	81	55	
150-795	F	28	January 18, 1933	198/130	56	22.5	72.6	97	78	53	
113-350	F	38	May 17, 1932	210/150	10	29.1	34.6	16	73	19	December 15, 1932
87-720	F	30	November 9, 1932	300/165	55	15.2	29.1	39	71	18	
135-051	F	11	January 12, 1933	275/151	30	20.1	34.5*	64	65	44	
119-162	F	55	November 8, 1932	235/155	40	16.5	13.7*	25	59	40	
150-053	F	56	November 29, 1932	200/100	70	19.6	16.4*	30	59	10	
113-019	M	13	June 28, 1932	150/90	10	23.1	15.8*	29	55	37	
138-277	F	19	January 2, 1911	170/95	10	20.5	40.1*	75	55	37	
			October 14, 1932	180/100	10	36.4	3.2*	6	7	5	
121-988	M	11	January 10, 1933	165/90	10	47.0	5.1*	9	12	8	
			July 16, 1932	190/125	55	20.3	10.3*	19	52	35	

TABLE IV (Continued)

Hospital number	Sex	Age	Date	Blood pressure	Phenol sulphon phthalein	Blood urea nitrogen	Urea clearance	Per cent of normal	Creatinine clearance	Per cent of normal	Date of death
		years		mm Hg	per cent in 2 hours	mgm per cent	cc per minute		cc per minute		
151-530	M	52	March 6 1933	220/30	50	22.8	23.4*	43	45	30	
141-842	M	56	June 28 1932	260/140	25	29.8	19.2*	36	45	30	
125-983	M	52	September 2 1932	170/120	40	17.4	28.1*	52	40	27	
144-079	F	38	October 11 1932	225/145	33	35.0	11.2*	21	35	24	
150-478	M	39	January 9 1933	220/135	25	40.6	15.4*	29	34	23	November 9 1932
142-341	F	38	March 14 1933	245/150	15	53.5	8.1*	15	13	9	
142-324	M	55	September 2 1931	170/116	5	18.0	25.6	34	32	22	
146-857	M	30	September 25 1931	240/140	5	74.1	29.2*	54	32	22	September 27 1931
			July 6 1932	220/160	10	109.0	0.7*	1	11	7	
148-705	F	48	July 23 1932	245/150	18	49.7	18.6	25	27	18	August 2 1932
149-634	M	44	August 31 1932	200/160	5	49.4	16.5	22	21	14	December 30 1932
149-237	M	58	November 8 1932	260/130	10	105.7	12.9	17	18	12	November 20, 1932
125-448	F	43	October 5 1932	150/90	10	57.4	5.2*	10	15	10	December 11 1932
			September 24 1932	160/110		76.3	17.5*	32	11	7	
150-929	F	60	October 13 1932	170/80		46.9	5.0*	9	7	5	January 23 1933
134-806	F	32	January 22 1933	230/140	5	156.0	3.8*	7	7	5	August 19 1931
144-535	F	45	July 22, 1931	230/110		61.5	4.1*	8	7	5	January 8, 1932
146-471	M	56	January 7 1932	160/100		131.5	1.7*	3	4	3	May 19 1932
91-504	F	43	May 19 1932	250/170	3	232.0	0.3*	0.6	3	2	June 18 1932
147-168	F	40	June 9, 1932	230/110	5	118.0	3.6*	7.0	3	1.4	June 29 1932
141-832	M	47	June 17 1932	235/130		88.9	1.7*	3.0	2	0.4	August 5 1931
			July 31 1931			163.0	0.2*	0.4	0.6		

* Standard clearances

TABLE V

Creatinine and urea clearances in patients with degenerative Bright's disease and certain other diseases

Hospital number	Sex	Age	Date	Diagnosis	Blood pressure	Phenol sulphon phthalein	Blood urea nitrogen	Urea clearance	Per cent of normal	Creatinine clearance	Per cent of normal	Date of death
		years			*mm Hg*	*per cent in 2 hours*	*mgm per cent*	*cc per minute*		*cc per minute*		
150-775	F	23	January 12, 1932	Degenerative Bright's disease and eclampsia	155/100	55	12.5	23.7*	41	56	38	
			March 20, 1933	Degenerative Bright's disease		42	13.7	31.3	42	77	52	
151-532	M	44	March 6, 1933	Degenerative Bright's disease	150/95		26.1	29.6	39	41	28	
151-037	F	21	February 1, 1933	Degenerative Bright's disease and eclampsia	165/120		60.4	17.5*	32	21	11	
			April 3, 1933	Degenerative Bright's disease	158/100	50	13.4	25.2*	47	61	41	
119-975	F	38	December 29, 1932	Diabetes and arteriosclerosis	150/90	35	27.3	33.0*	61	131	89	
119-703	M	53	January 21, 1933	Diabetes and arteriosclerosis	150/100	26	32.2	18.1	24	32	22	
0-320-284	M	43	January 14, 1933	Diabetes and arteriosclerosis	240/110		19.7	19.6*	36	51	34	
111-380	F	49	January 27, 1932	Diabetes and arteriosclerosis	230/130	40	9.1	12.0*	78	65	44	
136-525	M	28	October 25, 1930 / August 15, 1932	Diabetes insipidus / Diabetes insipidus	130/85		11.0 / 15.0	39.5 / 13.5	51 / 58	101 / 79	70 / 53	August 27, 1932

TABLE v (Continued)

Hospital number	Sex	Age	Date	Diagnosis	Blood pressure	Phenol sulphon phthalein	Blood urea nitrogen	Urea clearance	Per cent of normal	Creatinine clearance	Per cent of normal	Date of death
		years			mm Hg	per cent in 2 hours	mgm per cent	cc per minute		cc per minute		
133-845	M	23	August 22 1932	Essential Hypertension	160/110	80	8 1	103 0	137	119	80	
149-816	F	67	November 28, 1932	Carcinoma of stomach and arteriosclerosis	125/70		56 4	11 8*	22	44	30	November 30, 1932
149-535	M	64	December 5, 1932	Carcinoma of bronchus	110/70		15 0	21 1*	39	74	50	
122-258	F	66	June 10 1932	Carcinoma of stomach	170/90		6 5	14 5*	27	28	19	June 21, 1932
146-484	M	75	May 6 1932	Carcinoma of prostate	110/55		16 9	28 7*	53	81	55	May 9 1932
149-077	F	52	November 25 1932	Carcinoma of pancreas and jaundice	90/60	10	7 0	37 0*	69	20	14	November 30, 1932
146-070	M	50	April 7 1932	Pneumonia	110/65		19 2	63 5	85	88	59	September 14 1931
142-587	M	26	September 12 1931	Pneumonia	125/75		41 4	30 0*	56	145	98	September 27, 1931
142-828	M	36	September 25 1931	Pneumonia	130/70		15 0	62 0*	115	155	105	March 18, 1932
145-644	M	27	March 11 1932	Pneumonia	95/60		19 5	76 0*	141	231	156	January 13 1933
150-738	M	51	January 12 1933	Pneumonia	110/80		65 0	8 3*	15	38	26	November 18 1931
143-756	M	36	November 16, 1931	Pneumonia			183 0	9 45	13	23	16	December 18, 1931
144-053	M	71	December 8, 1931	Pyelonephritis	125/80	5	137 0	3 5	5	5	3	August 24 1932
147-549	F	34	July 19, 1932	Pyelonephritis	108/78	20	73 5	7 0*	13	9	6	March 18, 1932
85-756	F	70	March 13 1933	Pyelonephritis	130/76	Trace	175 0	0 8*	1 5	2	1 4	

TABLE V (Continued)

Hospital number	Sex	Age	Date	Diagnosis	Blood pressure	Phenol sulphonphthalein	Blood urea nitrogen	Urea clearance	Per cent of normal	Creatinine clearance	Per cent of normal	Date of death
		years			mm Hg	per cent in 2 hours	mgm per cent	cc per minute		cc per minute		
111–710	M	50	January 18, 1932	Bismuth poisoning and pyelonephritis	130/75	Trace	217 0	14*	3	2	14	January 28, 1932
113–816	F	39	November 30, 1931	Arteriosclerosis	180/140	80	7 8	68 0	91	147	99 0	
125–080	F	31	May 13, 1932	Arteriosclerosis	130/85	43	11 1	316*	59	157	106 0	
151–612	M	76	March 21, 1933	Hypertrophied prostate	140/80	85	17 6	37 8	50	68	16	
151–583	M	65	April 14, 1933	Hypertrophied prostate			16 5	42 6	57	68	16	
			March 21, 1933	Hypertrophied prostate	120/90		57 2	12 1	16	21	14	
			April 5, 1933	Hypertrophied prostate		40	32 3	27 3	36	41	30	
116–177	M	59	April 14, 1932	Pernicious anemia	115/55		10 8	44 8*	83	98	66	
150–871	F	38	February 14, 1933	Cirrhosis of liver and jaundice	110/70		14 5	39 4*	73	57	39	
111–512	M	50	March 15, 1933	Subacute endocarditis	160/90	15	30 6	24 3	32	24	16	
111–891	F	19	August 26, 1931	Streptococcic septicemia and acute interstitial nephritis	105/60	Trace	88 7	2 5*	5	3	2	September 8, 1931

* Standard clearances

standard urea clearance, however, may have a greater numerical value than the creatinine clearance determined at the same time, since the concentration ratio is multiplied by the square root of urine volume in the former and by the volume in the latter

A direct comparison of the two clearance tests is shown in Figure 2 in which the results of each test are plotted as percentage of average normal
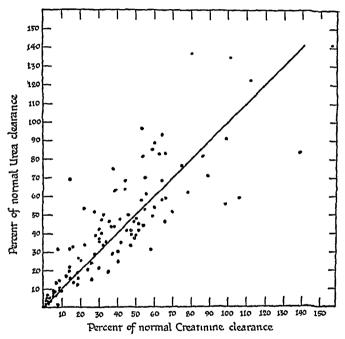
FIG 2 COMPARISON OF CREATININE AND UREA CLEARANCE TESTS IN PA-TIENTS WITH BRIGHTS DISEASE AND CERTAIN OTHER CONDITIONS

Points above the line indicate instances in which the urea clearance showed less reduction than creatinine, those below instances in which the percentage reduction in urea clearance was greater In patients with creatinine clearances below 10 per cent of average normal there is not much difference in the degree of reduction of the two tests In 82 creatinine clearances between 10 and 80 per cent of average normal, the urea clearance was reduced to a greater degree in 32, while in 50 it was not reduced to the same extent These differences are of questionable significance when the

TABLE V (Continued)

Hospital number	Sex	Age (years)	Date	Diagnosis	Blood pressure (mm Hg)	Phenol sulphon phthalein (per cent in 2 hours)	Blood urea nitrogen (mgm per cent)	Urea clearance (cc per minute)	Per cent of normal	Creatinine clearance (cc per minute)	Per cent of normal	Date of death
111-710	M	50	January 18, 1932	Bismuth poisoning and pyelonephritis	130/75	Trace	217 0	1 4*	3	2	1 4	January 28, 1932
113-816	F	39	November 30, 1931	Arteriosclerosis	180/110	80	7 8	68 0	91	147	99 0	
125-080	F	34	May 13, 1932	Arteriosclerosis	130/85	43	11 1	31 6*	59	157	106 0	
151-612	M	76	March 21, 1933	Hypertrophied prostate	110/80	85	17 6	37 8	50	68	16	
			April 14, 1933	Hypertrophied prostate			16 5	42 6	57	68	16	
154-583	M	65	March 21, 1933	Hypertrophied prostate	120/90	40	57 2	12 1	16	21	1 4	
			April 5, 1933	Hypertrophied prostate			32 3	27 3	36	1 4	30	
116-177	M	59	April 14, 1932	Pernicious anemia	115/55		10 8	11 8*	83	98	66	
150-874	F	38	February 14, 1933	Cirrhosis of liver and jaundice	110/70		14 5	39 4*	73	57	39	
111-512	M	50	March 15, 1933	Subacute endocarditis	160/90	15	30 6	21 3	32	24	16	
111-891	F	19	August 26, 1931	Streptococcic septicemia and acute interstitial nephritis	105/60	Trace	88 7	2 5*	5	3	2	September 8, 1931

* Standard clearances

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wide range of normal variation in both tests is remembered The most that can be said in favor of the creatinine test is that it may indicate a greater decrease in function more frequently than the urea clearance, but not with enough regularity to make up for the greater technical difficulty of the test Nor have we been able to find any group of patients or any pathological condition in which the results of one test are consistently different from those of the other

Since the creatinine test is more laborious and expensive, involving the ingestion of creatinine and the analysis of two blood samples, we do not believe it has any advantage in the routine estimation of the degree of impairment of kidney function in the clinic If it can be satisfactorily shown that the creatinine clearance does approximate the volume of glomerular filtrate, then a comparison of the two tests, run simultaneously, will permit a much more intimate analysis of the parts played by variations in the volume of filtrate and degree of back diffusion in health and disease (19)

CONCLUSIONS

The creatinine and urea clearance tests have been compared in normal persons and in patients with Bright's disease The mean creatinine clearance in 130 observations of 59 normal subjects was 148 cc per minute The variability of the two tests from the mean normal was approximately the same in our hands

In patients with Bright's disease the creatinine and urea clearance tests are generally equally reduced in relation to the average normal We were unable to demonstrate any practical advantage in the creatinine test to compensate for its greater technical difficulty

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EXPERIMENTS ON THE RELATION OF CREATININE AND UREA CLEARANCE TESTS OF KIDNEY FUNCTION AND THE NUMBER OF GLOMERULI IN THE HUMAN KIDNEY AT AUTOPSY

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Correlation of the results of tests of renal function with the pathological changes in the kidney has proved extremely difficult To cite a simple example in the marked proteinuria of degenerative Bright's disease, lesions of the glomeruli may be either slight or undiscoverable, while the tubule cells are strikingly abnormal And yet, by far the greatest part of the protein is believed to have been excreted through the glomeruli Similar difficulties have led many students to the conclusion that alterations of kidney function are independent of the nature of the anatomical changes in the kidney Fortunately for the clinician, the results of kidney function tests can be expressed in relation to the normal, without regard to the nature or the anatomical site of the condition which has produced the abnormality The average amount of urea excreted per minute by a normal individual with high urine volumes is equal to the urea contained in 75 cc of blood (1) If it is only equivalent to that contained in 20 cc of blood kidney function may be said to be reduced without any intimation whether this reduction is brought about by diminished blood flow decrease in the amount of renal tissue increased reabsorption of urea by the tubules or diminished secretion But an attempt to discover the mechanism of such reductions in function is irresistible to one concerned with the study of kidney disease

Van Slyke and his collaborators (2), from a comparison of " terminal " urea clearance values and autopsy findings suggested that in hemorrhagic and degenerative Bright's disease one may interpret the blood urea clearance as a measure of the proportion of glomerular tissue still functioning while in arteriolar sclerosis it appeared proportional to the decrease in renal blood flow rather than to the glomerular destruction Addis (3) used the ratio (urea in 1 hour's urine/blood urea concentration) (which under his conditions can be converted into maximum urea clearance by multiplying by 1 67), as a measure of the extent of the renal lesion—" the amount

little difference in the proportion of uninjected glomeruli   Any kidney in which a visible infarct was produced was discarded, or cut out and weighed and allowance made for it   It is assumed that the glomeruli are uniformly distributed throughout the suspension   Thorough mixing before sampling is essential   The variation in the counts of different aliquots is chiefly due to insufficient mixing   From 3 to 20 aliquots were counted, the standard deviation calculated and the estimate shown to be significant according to Fisher s (8) method of calculation[1]   It is further assumed that the numbers of glomeruli in the two kidneys of the same individual are equal within about 10 per cent   This was shown for the rabbit by Hayman and Starr (9), for the human by Moore (10)

Estimations of the number of glomeruli in the normal human kidney vary from 560 000 to 5,700,000 by different methods   By injection of iron salts and acid digestion Vimtrup (11) found 833,992 to 1,233,360 and Moore 800,000 to 1,000,000 with occasional values as low as 600 000 and as high as 1,200,000   Our series includes 12 kidneys from persons without histological evidence of significant abnormalities in the glomeruli or blood vessels   The counts ranged from 800,000 to 1 530,000   The mean was 1,156 000 and the standard deviation 191 266   This means that counts differing by more than twice the standard deviation, or below 773,000, probably indicate a significant decrease in the number of glomeruli, while those differing by more than three times the standard deviation or below 582 000 certainly do

Table I shows the comparison of creatinine and urea clearance tests with the estimated number of glomeruli in one kidney of 34 persons   We have followed Addis's classification of Bright's disease   In arteriosclerotic Bright's disease, decrease in clearances was associated with progressive reduction in the number of injected glomeruli   There were only 2 patients with active hemorrhagic Bright's disease and in one of these the estimate of the number of glomeruli is not significantly low   The other showed about the same reduction in clearance tests and glomeruli as the sclerotic group   Since in both chronic hemorrhagic Bright's disease and arterio sclerotic Bright's disease the patency of the renal vessels is reduced to

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[1] An example of the method of calculation   Hospital number 149–512   Weight of kidney before perfusion 163 grams   after perfusion 254 grams   Weight of pieces taken for histological section 2 grams   After digestion diluted to 16 liters   Counts on 2 cc aliquots 153 155 147 189 118 148, 154, 96 117, 111   Mean 138 8   Standard deviation 27   From these data, t calculated by the method of Fisher is 15 85 and the mean therefore significant. Estimate of total from mean 1,110 400   Correction for 2 grams taken for histological sections 8 812   Corrected estimate of count 1 119 212   In a count of 1 075 glomeruli from 3 blocks in sections so spaced that no glomerulus was counted twice, 1 052 contained injection mass   23 apparently patent glomeruli did not   Correction for uninjected patent glomeruli 24 465   Final estimate of patent glomeruli 1 143 400

of renal tissue which has been rendered functionless by the disease process "
He did not attempt to distinguish between actual decrease in mass of tissue
present and decreased accomplishment of a normal kidney mass   Thus
should the ratio be reduced, let us say half normal, by removal of one
kidney or by poisoning both kidneys even by a poison from which re-
covery could take place the two results would be considered the same—in
each half the renal tissue had ceased to work

Decrease in the creatinine clearance test has usually been ascribed to
decrease in the volume of glomerular filtrate (4)   Such a reduction could
be due to decrease in blood flow, blood pressure or filtering surface   On
the other hand, if the tubule cells were so damaged that they were unable
to resist back diffusion of creatinine filtered through the glomeruli, or to
secrete the constant fraction of the amount filtered as Shannon, Jolliffe
and Smith believe (5), there might be a reduction in creatinine clearance
without any decrease in the amount of glomerular filtrate   In such cir-
cumstance, it seems difficult to relate the decrease directly to reduction
in functioning mass of renal tissue

In the hope of furthering a physiological interpretation of the creatinine
and urea clearance tests, we have estimated the number of glomeruli in one
kidney of a number of patients whose clearances had been measured from
one day to several months before death   All of the patients had systolic
blood pressures above 100 mm Hg and were without evidence of congestive
heart failure at the time the clearance tests were made   When there was
any doubt of the ability to empty the bladder, the patient was catheterized
at the beginning and end of the period of urine collection   The tests were
made in the manner described in the preceding paper (6)

If there was any gross difference in the pathological condition of the
two kidneys, the experiment was discarded   The number of glomeruli
was estimated by Kunkel's (7) modification of Vimtrup's method   The
kidney was perfused at 140 mm Hg pressure with a mixture of equal
parts of 2 5 per cent potassium ferrocyanide and ferric ammonium citrate,
after washing out the blood with 0 9 per cent NaCl   It was then cut in
pieces, digested in 50 per cent HCl for 10 to 36 hours, washed in tap water,
and allowed to stand in distilled water for 24 to 48 hours   When suf-
ficiently digested, it was diluted to a suitable volume and thoroughly mixed
The number of glomeruli in 2 cc aliquots was then counted on a ruled
watch glass under a binocular microscope   In the majority of instances,
the completeness of injection has been checked by histological sections, and
the estimated number of glomeruli corrected for the proportion of ap-
parently patent but uninjected glomeruli

The use of such an estimate of the number of glomeruli involves several
assumptions   It is assumed that the proportion of uninjected glomeruli
is nearly the same throughout the kidney   Counts of injected and unin-
jected glomeruli in blocks taken from different parts of the kidney sho

little difference in the proportion of uninjected glomeruli Any kidney in which a visible infarct was produced was discarded, or cut out and weighed and allowance made for it It is assumed that the glomeruli are uniformly distributed throughout the suspension Thorough mixing before sampling is essential The variation in the counts of different aliquots is chiefly due to insufficient mixing From 3 to 20 aliquots were counted the standard deviation calculated and the estimate shown to be significant according to Fisher's (8) method of calculation[1] It is further assumed that the numbers of glomeruli in the two kidneys of the same individual are equal within about 10 per cent This was shown for the rabbit by Hayman and Starr (9), for the human by Moore (10)

Estimations of the number of glomeruli in the normal human kidney vary from 560,000 to 5,700 000 by different methods By injection of iron salts and acid digestion Vimtrup (11) found 833,992 to 1,233,360, and Moore 800,000 to 1,000,000 with occasional values as low as 600,000 and as high as 1,200 000 Our series includes 12 kidneys from persons without histological evidence of significant abnormalities in the glomeruli or blood vessels The counts ranged from 800,000 to 1,530,000 The mean was 1,156,000 and the standard deviation 191,266 This means that counts differing by more than twice the standard deviation, or below 773,000, probably indicate a significant decrease in the number of glomeruli, while those differing by more than three times the standard deviation, or below 582,000 certainly do

Table I shows the comparison of creatinine and urea clearance tests with the estimated number of glomeruli in one kidney of 34 persons We have followed Addis's classification of Bright's disease In arteriosclerotic Bright's disease, decrease in clearances was associated with progressive reduction in the number of injected glomeruli There were only 2 patients with active hemorrhagic Bright's disease and in one of these the estimate of the number of glomeruli is not significantly low The other showed about the same reduction in clearance tests and glomeruli as the sclerotic group Since in both chronic hemorrhagic Bright's disease and arteriosclerotic Bright's disease the patency of the renal vessels is reduced to

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[1] An example of the method of calculation Hospital number 149–512. Weight of kidney before perfusion 163 grams, after perfusion 254 grams Weight of pieces taken for histological section, 2 grams After digestion diluted to 16 liters Counts on 2 cc aliquots 153 155, 147 102 ' ' 148, 154 96 117, 111 Mean 138 8 Standard deviation 27 calculated by the method of Fisher, is 15 85 and the mean therefore Estimate of total from mean 1,110 400 Correction for 2 grams histological sections 8 812 Corrected estimate of 1,075 glomeruli from 3 blocks in sections so counted twice, 1 052 contained injection mass, 23 apparently patent did not Correction for uninjected patent glomeruli patent glomeruli 1 143,400

TABLE I

Relation of creatinine and urea clearance tests and the number of glomeruli in one kidney

Case number	Hospital number	Sex	Age	Diagnosis	Creatinine clearance	Urea clearance	Glomeruli in one kidney
			years		cc per minute	cc per minute	
1	143–651	F	34	Miliary tuberculosis	145	40 6*	1,260,000
2	149–586	F	23	Miliary tuberculosis	204	112 0	1,230 000
3	145–915	F	15	Tuberculous meningitis	129	66 7*	1,064,000
4	136–525	M	28	Diabetes insipidus	104	39 0	1,288,000
5	147–691	M	36	Miliary tuberculosis	108	36 0*	800,000
6	146–212	M	33	Tuberculous meningitis	94	51 0	896,000
7	145–644	M	27	Pneumonia	231	76 0*	1,250,000
8	142–828	M	36	Pneumonia	151	62 0*	1,037,000
9	142–587	M	26	Pneumonia	145	26 0*	1,336,000
10	150–738	M	51	Pneumonia	38	8 3*	1,530 000
11	143–756	M	36	Pneumonia	23	9 5	1,250,000
12	146–484	M	75	Carcinoma of prostate and arteriosclerotic Bright's disease	81	28 7*	1,056,000
13	149–816	F	67	Carcinoma of stomach and arteriosclerotic Bright's disease	44	11 8*	402,000
14	144–079	F	38	Arteriosclerotic Bright's disease	35	11 2*	490,000
15	145–997	M	29	Arteriosclerotic Bright's disease	47	27 0*	390 000
16	142–324	M	55	Arteriosclerotic Bright's disease	32	29 0*	174,000
17	149–143	M	54	Arteriosclerotic Bright's disease	30		341,000
18	143–018	F	25	Arteriosclerotic Bright's disease	27	12 3*	413,000
19	149–634	M	44	Arteriosclerotic Bright's disease	18	13 0	422,000
20	149–237	M	58	Arteriosclerotic Bright's disease	15	5 0	240,000
21	134–806	F	32	Arteriosclerotic Bright's disease	7	4 0*	359,000
22	91–504	F	43	Arteriosclerotic Bright's disease	3	3 6*	275,000
23	146–741	M	56	Arteriosclerotic Bright's disease	3	0 3*	168 000
24	147–168	F	40	Arteriosclerotic Bright's disease	2	1 7*	192,000
25	145–306	F	43	Arteriosclerotic Bright's disease	1		156,000
26	141–832	M	47	Arteriosclerotic Bright's disease	0 6	0 2*	195,000
27	149–512	M	21	Focal hemorrhagic Bright's disease	126	39 0*	1,143,000
28	141–891	F	19	Acute hemorrhagic Bright's disease	3	2 5*	819,000
29	142–730	M	36	Active hemorrhagic Bright's disease	29	13 8*	746,000
30	151–508	F	27	Active hemorrhagic Bright's disease	12	5 5*	170,000
31	144–740	M	50	Pyelonephritis with multiple abscesses and arteriosclerotic Bright's disease	2	1 4*	393,000
32	144–053	M	71	Hypertrophied prostate and pyelonephritis with multiple abscesses	5	3 5	490,000
33	147–594	F	34	Pyelonephritis and B coli septicemia	9	6 9*	1,330 000
34	149–077	F	52	Carcinoma of pancreas and bile nephrosis	3		645,000

* Standard clearance

about the same degree (12) and since not only is the final clinical picture often very similar but even the pathological distinction at times difficult, it seems that in both groups the reduction in clearances is probably due to reduction in number of glomeruli

In patients with acute nephritis who show decreased clearance values with return toward normal during convalescence it seems reasonable to suppose that certain glomeruli have been rendered temporarily functionless by inflammation, but that the process has not gone on to destruction so that recovery can take place   The one patient in the series with acute hemorrhagic nephritis had a very low clearance, but a normal number of glomeruli   The injection method of estimating glomeruli does not, of course, distinguish between normal and abnormal glomeruli since any whose capillaries are sufficiently patent to be injected at the pressure used must be counted   Sections of the other kidney from this patient showed degenerative or inflammatory changes in nearly every glomerulus

Möller, McIntosh and Van Slyke attribute decrease in the volume of blood cleared of urea per minute in pathological conditions to one of two causes either a decrease in the volume of blood passing through the kidneys, or a decrease in the proportion of its urea removed during the passage   In cardiac decompensation, glomerular nephritis and arteriolar sclerosis diminished flow seems certain   Whether a decrease in the proportion of urea removed from the blood also occurs they felt there was at present no basis to surmise   Holten and Rehberg on the other hand believe from comparison of creatinine and urea excretion rates that the proportion of urea removed is quite variable, the variation being due to back diffusion of different amounts of urea from concentrated solution in the lumen of the tubule   They believe that a certain amount of back diffusion takes place normally, and that this may be increased by tubular damage   The small group in Table I associated with general or urogenital infection lends support to this conception, but would extend it to include back diffusion of creatinine as well at least in pathological conditions This group showed low clearances and yet a normal number of glomeruli which in histological section did not appear significantly abnormal

While it is of course possible that the volume of glomerular filtrate may have been reduced in these cases it seems more probable that the cause of the low clearance values lies in toxic damage to tubule cells so that they are no longer able to resist back diffusion of urea or creatinine from the lumen of the tubule into the peritubular capillaries   This is illustrated in the group of patients with pneumonia   Cases 7 and 8 had normal concentrations of blood urea nitrogen and creatinine and normal clearances   Case 9 had a normal blood creatinine and creatinine clearance, but an elevated blood urea nitrogen and a reduced urea clearance   This we would interpret as indicating sufficient damage to tubules to permit an increase in the normal back diffusion of urea, but insufficient to allow

appreciable back diffusion of the normally more concentrated creatinine Cases 10 and 11 had increase of both urea nitrogen and creatinine, and a reduction in both clearances, but a normal number of glomeruli and adequate blood pressure   Here it seems probable that tubule cells were sufficiently damaged to permit back diffusion of both urea and creatinine We have no explanation to offer for the presence of this type of damage in some pneumonia patients and not in others

If this interpretation be correct its bearing on the significance of clearance tests is obvious They may be used as a measure of the "amount" of functioning renal tissue only in the absence of sepsis or toxic damage to tubules   When these are present, clearance tests indicate the manner in which the kidney eliminates the test substance, but how much of a reduced clearance is due to reduction in amount of functioning renal mass and how much to abnormal behavior of that mass it is impossible to say

Additional evidence that variation in the proportion of urea removed from the blood occurs is indicated by the effect of drugs (Table II)   After

TABLE II

Effect of drugs on creatinine and urea clearance tests

Name	Drug	Urine volume	Creatine clearance		Urea clearance	
		cc per minute	cc per minute	Per cent average normal	cc per minute	Per cent average normal
B	Control—1st hour	4 26	169	114	75 0	100
	Control—2nd hour	4 23	164	111	77 0	103
H	Before pituitrin	11 33	138	93	90 0	120
	After pituitrin	66	113	76	26 85*	50
C	Before pituitrin	8 75	86	58	49 5	66
	After pituitrin	3 17	71	48	24 9	33
S	Before adrenalin	7 70	196	132	75 0	100
	After adrenalin	3 75	139	94	57 5	77
B	Before morphine	4 80	181	122	87 0	116
	After morphine	2 75	143	97	82 7	110
L	Before caffeine	1 91	126	85	32 7*	61
	After caffeine	5 68	158	107	73 5	95

* Standard clearance

the administration of pituitrin, the excretion of urea is diminished relatively more than that of creatinine, while after adrenalin and morphine it is decreased less   After caffeine, there is a greater increase in urea clearance than in creatinine clearance   These experiments were all done in the

morning when MacKay (13) has shown that variations in clearances are least. Fluid intake was constant, the drugs given hypodermically five minutes before the end of the control hour. While there is nothing in the simple comparison of the two tests to indicate that it is the proportion of urea removed from the blood that varies, rather than creatinine, the relatively constant rate of creatinine excretion independent of urine volume, and the variation in the rate of urea excretion with urine volume make it seem more probable that there is greater variation in the proportion of urea removed than of creatinine. If the creatinine clearance represents the volume of glomerular filtrate, it must indicate that variations in the volume of filtrate are of less importance in determining the final volume of urine than are differences in the volume of fluid reabsorbed by the tubules.

SUMMARY

The results of creatinine and urea clearance tests of kidney function have been compared with estimates of the number of glomeruli in one kidney after postmortem injection. In arteriosclerotic Bright's disease and probably in chronic hemorrhagic Bright's disease reduction in clearance tests is associated with decrease in the number of injected glomeruli. In pneumonia and pyelonephritis, the clearances may be greatly reduced in the presence of a normal number of glomeruli. It is suggested that this may be due to back diffusion of the test substance through damaged tubule cells.

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SERUM TREATMENT OF HEMOLYTIC STREPTOCOCCUS PNEUMONIA

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During the several outbreaks of respiratory disease occurring during the colder months of the year the chance simultaneous occurrence of strep tococcus tonsillitis not infrequently results in hemolytic streptococcus lobu lar pneumonia Many of the cases of pneumonia seem to be secondary in the sense that the symptoms of an acute infectious disease such as influenza measles sinusitis etc. are present for a few days before the onset of pneumonia, whereas in a smaller number there occurs a sudden onset of lobular pneumonia usually with pleurisy without any evidence of a preceding infection The seriousness of both types of hemolytic streptococcus pneumonia with the high mortality and frequent occurrence of empyema is well known The accepted method of treatment is sup portive and symptomatic with surgical intervention whenever empyema occurs The only hope of successful treatment lies in the use of specific agents Since certain other streptococcal infections such as scarlet fever and erysipelas have apparently improved under the administration of spe cific antiserum it is logical to apply these methods to the streptococcal pneumonias At least one difficulty comes to mind immediately viz the problem of preparing a serum of sufficient polyvalency to be generally ap plicable The pathogenic hemolytic streptococci vary greatly in their anti genic values Even the strains isolated from cases of erysipelas and cel lulitis show considerable differences not only immunologically but in their fermentative reactions [1] However these strains by their breadth of im munological reaction are apparently interrelated and it has been found possible by means of the Lancefield precipitation test to select nine strains for the production of a polyvalent serum sufficiently broad to cover all the strains thus far recovered from cases of erysipelas and cellulitis This assumption of coverage is based on the Lancefield (1) M factor precipitin reaction in monovalent rabbit sera prepared against each of the nine strains on the neutralization of the toxallergin, on protection tests in mice and on clinical response in human cases

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[1] Unpublished experiments of Eleanor A Bliss

It has not been found possible to obtain the precipitin reaction with serum from immunized horses

fair number of white cells as well as albumin although the latter fell to a faint trace before discharge

She was discharged March 19 1930, six weeks after admission
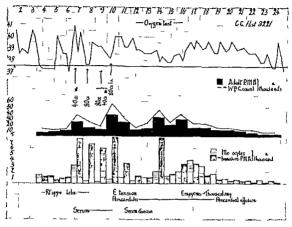
CHART I   CASE II

Case II   C C   male aged twenty two years was well until February 2 1931 when he had a sudden attack of high fever headache weakness nausea and vomiting and was admitted to the infirmary   Recovery was uneventful and he was discharged on February 7th   He was extremely weak and had severe night sweats but returned to school and work on February 12th   One week later he was readmitted to the infirmary with German measles and remained four days   He did not return to work until February 24th   On the evening of February 28th there was a sudden onset of high fever accompanied by aching and he was readmitted to the infirmary on March 1st   For three days the temperature varied from 37 to 40 5° C   with two chills each day   On March 5th he was admitted to the hospital with signs of lobular pneumonia in the right upper lobe   The sputum culture showed innumerable colonies of β hemolytic streptococci   The temperature fluctuated between 37 and 41° C   On the sixth day after onset the patient had two chills seemed much worse and complained of severe pain in the right side of his chest   In the afternoon 40 cc of concentrated erysipelas serum were injected intramuscularly and in the afternoon of the seventh day 80 cc of the unconcentrated serum were given intravenously   These doses were repeated on the eighth day and the intravenous injection repeated again on the tenth day when there were signs of extension to the right lower lobe   During the four days of serum treatment the immature polymorphonuclears increased but decreased on the eleventh day   The fever became continuous instead of remittent   On the twelfth day there were signs of pericarditis and on account of the extreme cyanosis the patient was placed in the oxygen tent for four days   Fluid was detected in the right pleural cavity

The first opportunity to apply this serum in the treatment of strep tococcus pneumonia came before any immunological tests were carried out. But such tests coincidently with treatment were carried out showing that the polyvalent serum possesses antibodies for the particular strain involved.

The purpose of this report is to record the details of the treatment of eight successive cases[4] of β-hemolytic streptococcus pneumonia all of whom recovered.

Case I I D, female aged twenty one years was admitted February 14 1930 complaining of chills fever and swollen cervical lymph nodes of nine days' duration. Past history. Frequent tonsillitis before tonsillectomy twelve years ago and a rather severe sinusitis one and a half years ago.

The illness for which she was admitted began simply enough as a sore throat and head cold with rather marked swelling of the lymph nodes and later fever.

For a week the temperature was normal every morning, reaching 39.5° and 40° C at night with profuse sweats and later with pain over the right side of the face. On admission she appeared moderately ill and showed little except the red pharynx the tenderness over the right maxillary frontal sinus and general moderate lymph node enlargement. Her temperature was 39.7° C, pulse 138, blood pressure 130 systolic and 80 diastolic, her leukocytes were 17 600 with 54 per cent polymorphonuclears. The urine showed red and white cells and 2 + albumin.

At this time she was found to have an acute right maxillary sinusitis and a mild acute nephritis. Despite puncture of the antrum four days later her temperature continued to fluctuate widely from 36.5° to 40.5° C in the course of six hours. The urine contained red cells and albumin and the nonprotein nitrogen rose to 48 mgm per cent. β-hemolytic streptococci were grown from the antrum washings. On the twenty-second day after onset she developed signs of pneumonia of the right upper lobe. Cultures and mouse inoculation of the washed sputum showed predominance of β-hemolytic streptococci which by the Lancefield method yielded a specific soluble substance precipitated by mono valent antierysipelas serum. She was given two intravenous injections of anti erysipelas serum first 25 cc and then 50 cc. Following this the temperature fell to normal and the excretion of urine rose from 560 cc per day (average for seven days) to 2340 cc the following day and continued at an average of 1460 cc per day.

Her course was then uneventful except for two moderate attacks of serum sickness. She began to gain in weight and strength. Her red count and hemo globin began to return to normal. Her sinuses cleared and showed no further streptococci on culture although her pharynx continued to harbor a decreasing number of these organisms. On the day of discharge a culture was negative.

The only abnormality was in the urine. The kidney function tests were normal but there were continuously present great numbers of red cells and a

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[2] Polyvalent antistreptococcus hemolyticus serum (unconcentrated) and a concentrated product (erysipelas antitoxin) prepared by H K Mulford Com pany using nine selected strains. In two cases streptococcus antitoxin prepared by the New York State Laboratory as used in addition.

[4] Abstract of the first five cases treated as presented before the Association of American Physicians in 1931 by Amoss H L Persons E I and Hall H E Price O C
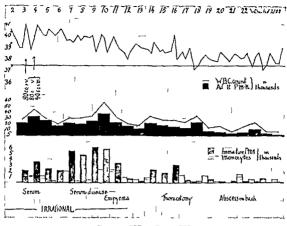
CHART III   CASE IV

Case IV W W male aged fifteen years about January 15 1931 ex
perienced a soreness in his throat and the following day was admitted to the
infirmary where he was placed in the same room with several patients ill with
influenza About January 30th he had a chill and became very ill Signs of
pneumonia over the entire right side of the chest appeared on February 1st and
he was transferred to the Medical Service By February 2 1931 the sputum
contained pus and many β hemolytic streptococci The x ray showed diffuse
peribronchial reaction throughout the right lobes thickened pleura and a shadow
obscuring the outer portion of the diaphragm The patient was irrational the
temperature varied from 39 to 41 C and the blood pressure was 80 systolic
and 40 diastolic Eighty cubic centimeters of erysipelas antistreptococcus
serum were given intravenously and the treatment repeated the following day
with 40 cc of the concentrated serum intramuscularly The temperature which
had fluctuated markedly began to be continuously high and gradually to recede
Serum disease appeared on the seventh day and the immature polymorpho
nuclears increased One hundred cc of fluid were withdrawn from the right
pleural cavity on the tenth day but tidal irrigation was not begun until the fif
teenth day Cultures of fluid showed heavy growth of β hemolytic strepto
cocci The Schilling count shifted to the right and there was a gradual and
complete recovery The patient was discharged well on April 4 1931

Cases treated by intrapleural injection of serum

In three of the four cases already described empyema developed re
quiring long drainage In the two cases to be described the serum was
injected into the pleural cavity with a twofold purpose (*a*) as a mechanical
method of affording relief from severe pleurisy and (*b*) to prevent if
possible empyema In these cases the intravenous and intramuscular in
jections were also given

on the eighteenth day. One liter of pus was evacuated and tidal drainage insti-
tuted. Culture of the empyema fluid showed heavy infection with β hemolytic
streptococci. On the nineteenth day the patient expectorated about 200 cc of
sputum and the signs of pericardial effusion disappeared within two days. On
the twenty-fourth day the roentgen ray showed signs of cavitation in the right
upper lobe and there was continuous expectoration of pus which contained
many spirochetes and β-hemolytic streptococci. After postural drainage and
the intravenous injection of neoarsphenamine the lung abscess gradually healed
and the patient gradually improved. He was discharged well on September
30, 1931.

CHART II   CASE III
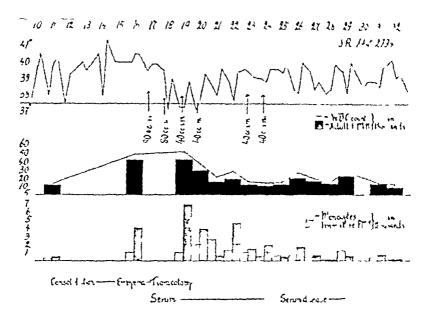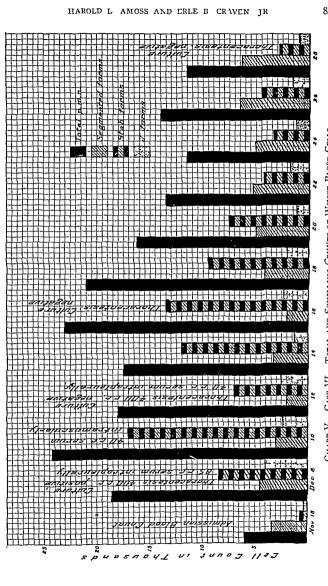
Case III J R male aged sixteen years was admitted January 27 1931
from the infirmary on the tenth day of a very severe laryngitis with a high
fever and unproductive cough. On the eleventh day there were signs of con-
solidation in the left lower lobe. On the fifteenth day there were signs of fluid
at the left base and cultures of the sputum taken on this date showed innumerable
β-hemolytic streptococci. On the sixteenth day there was a rise in the imma-
ture polymorphonuclear cells in the blood and fluid containing large numbers
of pus cells (culture positive for β hemolytic streptococci) was removed from
the pleural cavity. On the seventeenth day the patient became much worse.
On this day 80 cc of erysipelas anti-streptococcus serum were given intra-
venously and the dose repeated the following day and tidal drainage of the
pleural cavity was begun. On the succeeding six days there were given intra-
muscular injections of 40 cc each of the concentrated serum intramuscularly. Then the num-
ber of immature polymorphonuclear cells began to recede on the twenty-third
and the patient began to improve. Convalescence was slow, but the patient was
discharged well on May 15 1931.

CHART V CASE VI TOTAL AND SCHILLING COUNTS OF WHITE BLOOD CELLS
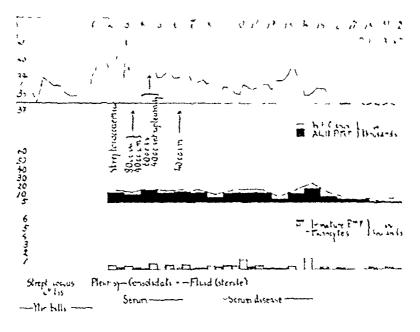
CHART IV   CASE V

As evidence that the effect of the serum is a result of specific action rather than a non specific provocation of reticuloendothelial cells (2) two cases are described below in which the pleural fluid contained in addition to the β hemolytic streptococcus a strain of pneumococcus Group IV After adequate doses of the serum streptococci were no longer present but the pneumococci endured

Case VII A six year old colored female was admitted to the Pediatric Ward [5] on January 11 1933 on the seventh day of lobular pneumonia and with signs of fluid in the left pleural cavity The temperature was 40 5° C and white blood cells 20 500 per cubic millimeter Sputum yielded pure culture of β hemolytic streptococci and the blood culture showed less than one colony per cc of the same strain Thoracentesis immediately after admission yielded a small quantity of sanguineous fluid which on culture, had a heavy growth of β hemolytic streptococci The patient grew progressively worse On January 15th and 16th 15 and 40 cc of fluid respectively were withdrawn and cultures were heavily positive On January 17th following aspiration of 30 cc of fluid 10 cc of erysipelas antitoxin were instilled into the pleural cavity There was no reaction On January 20 1933 40 cc of erysipelas antitoxin were injected intrapleurally following aspiration of 280 cc of pleural exudate Culture of this aspirated fluid again yielded β hemolytic streptococci Cultures of fluid removed on January 25th and 27th were negative for hemolytic streptococci but gave a heavy growth of pneumococcus Group IV Before serum was given the temperature reached daily peaks of 39° to 40 6° C and the white blood count 25 000 Following the serum injections the white blood count dropped rapidly to 12 000 and the peaks of fever to 38 5° to 37 2° C After thoracenteses on January 25th and January 27th (yielding Group IV pneumococci) the temperature again rose to 40 6° C

Thoracotomy for the purpose of tidal irrigation was performed on January 30 1933 Pus obtained at operation gave a pure culture of pneumococcus Group IV

Sputum culture now gave a mixed growth of pneumococcus Group IV staphylococcus aureus and β hemolytic streptococci Blood cultures remained sterile

Four days after operation the temperature fell to 37 6° C and remained normal until March 13th when the drainage tube was removed There was an immediate rise in temperature to 39 3 C and the child became quite ill again Drainage was re instituted and the temperature came to normal after six days At discharge March 24 1933 the thoracotomy wound had healed and the child was discharged in excellent condition

Case VIII Colored male aged four years brother of patient described under Case VII admitted on January 11 1933 on the fifth day of lobular pneumonia of left lung with pleurisy thickened pleura and early empyema Temperature 40 2 C white blood cells 11 600 sputum yielded a heavy growth of β hemolytic streptococci and the blood culture showed less than one colony per cc of the same organism Thoracentesis on the day of admission yielded a small quantity of sanguineous fluid which on culture had a heavy growth of β hemolytic streptococci

hazy. The temperature became normal on February 15th and he made an uneventful recovery save for severe serum sickness. The patient was discharged well on March 14th.

Case II. White male, aged forty-eight years, admitted on February 1, 1932, with the diagnoses of pernicious anemia and subacute combined sclerosis of spinal cord. He was treated with liver and liver extract and was discharged improved on February 14th.

He continued to take liver extract irregularly and was readmitted on November 18, 1932, with more advanced spinal cord changes and a neurological bladder. There had been only a moderate diminution in the erythrocyte count and hemoglobin.

On the eleventh day after admission his temperature rose to 39° C. and the leukocyte count to 14,500. Examination disclosed nothing to account for the fever. The irregular fever rising to 38.5° to 39.5° C. daily continued and on December 7th the patient complained of severe pain in the right flank and down the lower right anterior costal margin. Respirations were shallow and there was diminished excursion on the right. Tactile tremitus was moderately increased from the angle of the scapula to the right base. The percussion note was slightly impaired and a loud rough friction rub obscured the breath sounds. On the following morning the breath sounds were tubular and the friction rub was still present. The patient was acutely ill and was very uncomfortable. Culture of the sputum showed heavy growth of β hemolytic streptococci. With the appearance of the fluid at the right base, the audible rub disappeared but the severe pain endured. The blood culture taken on December 8th showed one colony of β-hemolytic streptococci per cubic centimeter. On December 9th from the right pleural cavity, 400 cc. of thin bloody fluid were withdrawn which on culture yielded a heavy growth of β hemolytic streptococci. Following thoracentesis 85 cc. of concentrated erysipelas streptococcus antitoxin diluted with 85 cc. of physiological saline were injected directly into the pleural cavity without immediate reaction. There was no increase in the pain.

On December 10th 40 cc. of the antitoxin were given intramuscularly. On December 11th 500 cc. of pleural fluid were again withdrawn and 40 cc. of concentrated streptococcus antitoxin injected. The fluid withdrawn was no longer bloody and cultures remained sterile. The leukocyte count of 37,000 on the day before injection of serum fell to 24,000 on the following day and to 17,000 on the third day (see Chart V). The fever which during the height of the disease ranged between 38.5 and 40° C. now fluctuated between 38 and 38.8° C. The patient looked and felt much improved although signs of considerable pleural effusion remained. The irregular fever continued. Thoracenteses on December 17th and 29th and January 13, 1933 yielded quantities of fluid from 40 to 500 cc. All were cultured and remained sterile. The temperature gradually fell to normal during the five weeks following serum injection. The patient was discharged on January 26, 1933 having been afebrile for two weeks but with evidence of a small amount of encapsulated fluid along the right lateral chest wall.

Specific action of the serum

In Case V it is possible that the intrapleural instillation of serum prevented infection of the pleural fluid by streptococci and in Case VI the serum apparently assisted within forty-eight hours in the removal of the growth already established.

As evidence that the effect of the serum is a result of specific action rather than a non specific provocation of reticuloendothelial cells (2) two cases are described below in which the pleural fluid contained in addition to the β-hemolytic streptococcus a strain of pneumococcus Group IV After adequate doses of the serum streptococci were no longer present but the pneumococci endured

Case VII A six year old colored female was admitted to the Pediatric Ward⁵ on January 11 1933 on the seventh day of lobular pneumonia and with signs of fluid in the left pleural cavity The temperature was 40 5° C and white blood cells 20 500 per cubic millimeter Sputum yielded pure culture of β hemolytic streptococci and the blood culture showed less than one colony per cc of the same strain Thoracentesis immediately after admission yielded a small quantity of sanguineous fluid which on culture had a heavy growth of β hemolytic streptococci The patient grew progressively worse On January 15th and 16th, 15 and 40 cc of fluid respectively were withdrawn and cultures were heavily positive On January 17th following aspiration of 30 cc of fluid 10 cc of erysipelas antitoxin were instilled into the pleural cavity There was no reaction On January 20 1933 40 cc of erysipelas antitoxin were injected intrapleurally following aspiration of 280 cc of pleural exudate Culture of this aspirated fluid again yielded β hemolytic streptococci Cultures of fluid removed on January 25th and 27th were negative for hemolytic streptococci but gave a heavy growth of pneumococcus Group IV Before serum was given the temperature reached daily peaks of 39 to 40 6 C and the white blood count 25 000 Following the serum injections the white blood count dropped rapidly to 12 000 and the peaks of fever to 36 5° to 37 2° C After thoracenteses on January 25th and January 27th (yielding Group IV pneumococci) the temperature again rose to 40 6 C

Thoracotomy for the purpose of tidal irrigation was performed on January 30 1933 Pus obtained at operation gave a pure culture of pneumococcus Group IV

Sputum culture now gave a mixed growth of pneumococcus Group IV, staphylococcus aureus and β hemolytic streptococci Blood cultures remained sterile

Four days after operation the temperature fell to 37 6° C and remained normal until March 13th when the drainage tube was removed There was an immediate rise in temperature to 39 3 C and the child became quite ill again Drainage was re instituted and the temperature came to normal after six days At discharge March 24 1933 the thoracotomy wound had healed and the child was discharged in excellent condition

Case VIII Colored male aged four years brother of patient described under Case VII admitted on January 11 1933 on the fifth day of lobular pneu

Skin neutralization tests [6]

The streptallergin from four strains (Cases II to V) gave a positive skin reaction in goats   The addition of 0 1 cc of serum from a goat immunized with the New York 5 scarlet strain of β hemolytic streptococcus prevented any reaction in the presence of 10 skin test doses of the streptallergins

Protection tests [7]

The virulence of four of the strains was increased by mouse passage until the minimum lethal dose became 0 5 cc of 10 ^{7} dilution

The protection tests were made by injecting intraperitoneally 0 2 cc of the anti serum diluted to 0 5 cc with isotonic salt solution and simultaneously but separately 0 5 cc of the dilution of the eighteen hour broth culture of the organism   Three mice were used for each dilution and a virulence control and normal horse serum control were injected in each series

The results of the protection tests were as follows

Case I   Not tested

Case II   Virulence 10 ^{6} Mulford s concentrated protected against 10^{-r} unconcentrated   result inconsistent, New York State no protection

Case III   Virulence 10^{-6}, Mulford s concentrated protected against 10^{-5} unconcentrated   result inconsistent   New York State no protection

Case IV   Virulence 10^{-6} Mulford s concentrated protected 2 out of 3 against 10^{-4}, unconcentrated protected against 10$^-$, New York State no protection

Case V   Control 2 out of 3 mice died after injection of 10 dilution plus normal horse serum   Mulford s concentrated streptococcus antitoxin protected all mice against 10 ^{3} New York State antistreptococcus serum protected 2 mice out of 3 against 10$^-$

Cases VI, VII and VIII   strains *avirulent* for mice

Protection tests with convalescent serum from the patients have not yet been made

DISCUSSION

The patients treated with serum were critically ill   With the high fever cherry cyanosis hypotension distension and evidences of a rapidly spreading lesion the prognosis was very grave   In Case I the administration of the serum was followed by a marked increase of urinary secretion and quick disappearance of fever with a definite improvement in the

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[6] These tests were made by Miss Ruth Wheeler of the New York State Laboratory of Hygiene to whom we are greatly indebted for her cooperation

Method of Mr C Roos Glenolden Pa

mouse protection tests suggest a rational immunologic basis for specific serum treatment

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CYSTINE CONTENT OF FINGER NAILS IN PELLAGRA [1]

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An abnormality in sulfur metabolism in pellagra was first demonstrated by Camurri (1) and Myers and Fine (2) Indicanuria (3, 4), a decreased excretion of thiocyanate (5), and an increase in the excretion of absolute amounts of ethereal sulphates in the urine (2) of pellagrins have been observed A reduction of sulfur in brain and spinal cord of five individuals succumbing with pellagra was reported by Koch and Voegtlin (6) The total amount and per cent cystine of rat hair decreases when these animals are fed diets deficient in vitamin G (7, 8)

It has been suggested (9) that the relatively high concentration of cystine in wool and hair is of physiologic importance in the protection of the organism against harmful effects of prolonged exposure to sunlight The protecting effect of cystine solutions to paramecia exposed to ultraviolet rays was demonstrated by Harris and Hoyt (10) and experiments of Ward (11) seem to indicate that although several amino acids give marked general absorption bands, cystine is the only amino acid that has any marked absorption in the ultraviolet region of solar light The high concentration of cystine in epidermal tissues of higher forms of animal life (12 13) a content greater than that of any other tissue, suggests a possible specific protective function in this tissue In the hope that we might find more experimental evidence for the clinical observation (14 15) that skin lesions in pellagra indicate a light sensitive condition 40 cases of pellagra and 61 subjects without evidence of pellagra have been studied Because of the technical difficulties in obtaining representative samples of skin we concluded that finger nails gave more uniform data from which to judge cystine content in epithelial tissue

METHODS

In reviewing the available data concerning the sulfur and cystine content of the common keratins one is impressed with the wide variation of the results in the hands of different observers Much of the data is open to criticism in that no mention is made concerning the method of preliminary treatment and variations in this may account for many of the discrepancies Our preliminary work showed that if nails are thoroughly cleaned and the technique of drying standardized there is little variation in cystine in nails from various fingers of the same individual However in spite of an attempt to subject each sample of

of 9 7 to 11 7 per cent   Sullivan and Hess (23), in 1932, reported cystine content of the finger nails of normal individuals to vary from 11 to 13 per cent   Since the early methods involved the isolation of cystine from a hydrolysate and determination of precipitated sulfate, one would expect higher values in a colorimetric method such as Sullivan's

In our analyses of finger nails from 36 healthy young adults, chiefly medical students and laboratory workers, Figure 1, *A* and Table I, cystine
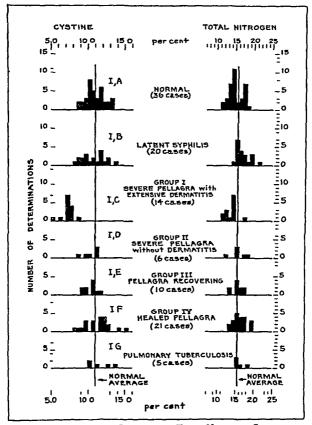
FIG 1   HISTOGRAMS FOR CYSTINE AND TOTAL NITROGEN CONTENT OF THE FINGER NAILS IN NORMAL SUBJECTS AND IN PATIENTS WITH PELLAGRA SYPHILIS AND TUBERCULOSIS

Each square represents a single determination

nail parings to standard conditions of cleaning and drying it was realized that some variation in the rate of loss of water from different samples was unavailable Therefore the total nitrogen content (representing protein) and the N S ratio were determined in a further effort to minimize the errors inherent in a series of determinations based on the dry weight of biological material

Finger nail parings were soaked in water for 24 hours, cuticle and dirt removed by scraping and after washing in running water dried in an oven for 24 hours at 90° C Cystine was determined by a modification of the Sullivan method (16) From 30 to 60 mgm clean dry finger nail parings and 5 cc of 20 per cent hydrochloric acid were placed in a 50 cc acetylation flask with a ground glass fitted reflux condenser The flask was placed in a cold mineral oil bath and the bath brought to 120° C at which heating was continued for 12 hours The hydrolysate was transferred to a 25 cc volumetric flask and the acetylation flask rinsed with 5 cc of 0 1 N hydrochloric acid The hydrolysate was treated drop-wise with 20 per cent sodium hydroxide until greenish to bromphenol blue, i e , about pH 3 5 Dilution was made to 25 cc with addition of 0 1 N hydrochloric acid Colorimetric estimation of cystine proceeded according to Sullivan s method Color matching is facilitated by the use of a yellow light filter glass disc devised for the Folin and Malmros (17) micro method for blood sugar determination [2]

Total nitrogen of finger nails was determined by the Koch-McMeekin method (18) on the above hydrolysate

Because of the slow and variable growth of the finger nail only cases with chronic disease have been considered and only stable conditions of at least two months duration have been included in the data Thirty-three of the pellagrins were negro patients at the State Hospital at Goldsboro, North Carolina,[3] and were carefully followed for months Several had experienced "recurrent" pellagra for years and had been observed at the institution over long periods of time All gave histories of faulty dietaries for months, and three mental cases were observed to develop symptoms during self-inflicted periods of starvation Treatment in all cases was chiefly dietary and followed the suggestions of Goldberger et al (19, 20) High vitamin foods, especially milk and fresh meats, fruits, and vegetables, were given in abundance Since many of the pellagrins were known to have syphilis on the basis of history of infection or positive blood Wassermann tests, data are presented from a control group of 20 cases with latent syphilis Also since emaciation is common among pellagrins five malnourished cases of pulmonary tuberculosis are included

Cystine content of normal finger nails

Only a few values for the cystine or sulfur content of normal human finger nails are available The early work of Mulder (21), in 1847, showed human nail to contain 2 8 per cent sulfur corresponding to 10 5 per cent cystine Buchtala (13), in 1907, pooled the finger nails from 16 cadavers and by a method which determined sulfur by sublimation, estimated cystine content to be 5 15 per cent Langecker (22), in 1921 reported values for sulfur in finger nails from six patients with various diseases His values range from 2 59 to 3 12 per cent corresponding to estimated cystine values

TABLE II

Cystine, total nitrogen and N S ratio in finger nails of individual negro cases of severe pellagra with extensive dermatitis before and after recovery

Case number	Sex	Age	Cystine	Total nitrogen	N S ratio*	Clinical notes
		years	per cent	per cent		
101	F	50	7 04	16 0	8 51	Extensive dermatitis Died 2 weeks later
107	F	60	7 46	15 8	7 94	Severe dermatitis Later recovered
108	F	63	8 55	15 4	6 76	Severe dermatitis Died 4 weeks later
109	F	53	7 52	12 6	6 27	Extensive dermatitis
			11 80	15 7	4 98	4 months after recovery
111	F	32	7 69	13 8	6 74	Severe dermatitis Later re covered
112	F	30	7 50	13 8	6 90	Extensive dermatitis
			10 90	17 9	7 43	6 months after recovery
113	F	29	7 32	13 1	6 71	Severe dermatitis
			8 22	16 3	7 41	3 months after recovery
114	F	46	7 27	12 6	6 50	Severe dermatitis
			10 83	13 7	4 74	5 months after recovery
115	F	45	8 00	15 4	7 20	Extensive dermatitis Died 9 weeks later
118	M	17	7 09	15 0	7 98	Severe dermatitis
			10 72	14 3	5 00	6 months after recovery
119	M	70	14 49	19 2	4 95	3 months before onset of symptoms
			7 85	14 5	6 90	Severe dermatitis
120	M	32	5 03	15 1	11 28	Most severe dermatitis
			9 53	17 8	7 01	4 months after slow improvement
127	M	27	6 15	18 1	11 01	Very severe dermatitis Later recovered
140	M	40	7 24	15 7	8 14	Severe dermatitis Later re covered

14 cases severe dermatitis

	Cystine	Total nitrogen	N S ratio*	
Minimum	5 03	12 6	6 27	
Maximum	8 55	18 1	11 27	
Average	7 26	14 8	7 63	

5 cases following recovery

	Cystine	Total nitrogen	N S ratio*	
Minimum	8 22	13 7	4 74	
Maximum	11 08	17 9	7 43	
Average	10 49	15 6	5 91	

* S calculated from cystine

TABLE I

Summary of cystine, total nitrogen and N:S ratio in finger nails

	Number of cases	Cystine			Total nitrogen			N:S ratio*		
		Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
		per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Normal	36	8.77	13.38	10.97	12.6	18.7	15.8	4.61	5.92	5.40
Pellagra										
Group I Severe with dermatitis	14	5.03	8.55	7.26	12.6	18.1	14.8	6.27	11.27	7.63
Group II Severe without dermatitis	6	8.93	11.98	10.61	12.6	18.3	16.2	4.71	6.17	5.71
Group III Healing	10	9.26	11.93	10.47	13.5	17.6	15.5	1.57	6.16	5.56
Group IV Cured	21	8.22	15.42	11.32	13.6	19.8	16.3	4.53	7.13	5.40
Syphilis	20	8.20	13.55	10.64	14.1	21.9	16.9	4.81	7.11	5.95
Tuberculosis	5	10.28	13.64	11.66	15.1	18.8	16.3	4.63	5.91	5.25

* S calculated from cystine

varies from 8.77 to 13.38 per cent with an average of 10.97 per cent and total nitrogen varies from 12.6 to 18.7 per cent with average of 15.8 per cent. The N:S ratio averages 5.40 and remains relatively constant with extremes of 4.61 and 5.92. The greater range of cystine in our group compared to that of Sullivan, using essentially the same method, may possibly be explained on the basis of variation in selection of individuals for the " normal " group.

Cystine content of finger nails in pellagra, syphilis, and pulmonary tuberculosis

The pellagrins are arranged into four arbitrary groups. Group I, severe pellagra with extensive dermatitis, Group II, severe pellagra without dermatitis, Group III, pellagra during recovery, and Group IV, completely healed pellagra. An exfoliative dermatitis of the hands, arms, and feet and in most cases of the legs and thighs had been apparent in the 14 cases in Group I for at least two months. All cases were extremely ill at the time of the determination and Cases 101, 108, and 115 (see Table II) died with inanition soon after the observation. A stomatitis was common to all and several had in addition diarrhea, psychosis, or dementia. In this group (see Figure 1, C and Table I) cystine is greatly reduced averaging 7.26 per cent. The highest result, 8.55 per cent, falls below the lower limit of the normal range. In Cases 120 and 127 (see Table I) in which

lagra during which time the patient remained on an adequate diet containing an abundance of all vitamins   Thirteen had recovered from severe pellagra with extensive dermatitis, four from severe pellagra of the gastro intestinal or central nervous system type in which dermatitis had been absent or played a minor role, and four from mild diarrhea and slight dermatitis   In this group (see Figure 1 F and Table I) cystine varies from 8 22 to 15 42 per cent   Two results, 14 49 per cent and 15 42 per cent, lie above the upper normal limit   Total nitrogen varies from 13 6 to 19 8 per cent also showing a greater variation than normal in the upper limit   Both the average cystine and total nitrogen approximate the average of the normal group

The group of 20 luetics consists of patients treated for at least two months at the syphilis dispensary of the Duke Hospital, and although at the time the analyses were made, none had active lesions, all had a history of luetic infection and the blood Wassermann test had been four positive The group is representative of widely divergent dietaries as observed in mill hands, farmers, shopkeepers etc in the pellagra belt   No definite deficiency could be ascribed to any diet although several of the patients were obviously malnourished and many consumed large amounts of corn and fat pork to the exclusion of fruits and other vegetables and meats Cystine varies more widely in this group (see Figure 1 and Table I) than in the normal, 8 20 to 13 55 per cent, in three cases the result falling below the lower limit of normal and in one case above the upper limit   However, the average for the group 10 64 per cent, approximates the normal average Total nitrogen tends to be increased varying from 14 1 to 21 9 per cent and averaging 16 9 per cent, thereby increasing the average N S ratio to 5 95

The 5 cases of pulmonary tuberculosis gave histories of loss of 20 to 60 pounds of weight during the preceding months and were obviously malnourished, judging by the severe emaciation   Cystine and total nitrogen tend to be above normal in these cases (see Figure 1, G and Table I), cystine ranging from 10 28 to 13 64 per cent and averaging 11 66 per cent and total nitrogen from 15 1 to 18 8 per cent and averaging 16 3 per cent   Because of the concomitant rise of both cystine and total nitrogen the N S ratio values fall within normal limits

DISCUSSION AND SUMMARY

The foregoing results support the opinion that there is an abnormality of sulfur metabolism in pellagra which is reflected in epithelial tissue and specifically related to the dermatitis of this disease   A study of the data from 36 normals and 20 cases of syphilis shows no constant variation of cystine with difference in sex race, or age   Since cystine was reduced and N S ratio increased in the finger nails from all of the 14 cases of pellagra with severe dermatitis, whereas in all cases of pellagra without

dermatitis was most extensive and severe, cystine content is the lowest, 5 03 per cent and 6 15 per cent   A determination three months before the appearance of dermatitis and diarrhea in Case 119 (see Table I) is of particular interest since at that time the cystine content was 11 49 per cent   At this time, because of emaciation, the patient was taking a diet rich in vitamins and calories   Later he refused food and in three month developed a severe dermatitis   Six months after the original observation cystine had decreased to 7 85 per cent, a reduction of 46 per cent   In five cases results were obtained following recovery (see Table II)   Cystine had returned to normal limits in all cases and at this time the average cystine, 10 49 per cent, was but slightly below the average for the normal group   Total nitrogen remained practically unchanged varying from 12 6 to 18 1 per cent with an average of 14 8 per cent   The reduction in cystine results in a relative increase in the N S ratio

Group II, severe pellagra without dermatitis, consists of six cases   four extremely emaciated, weak, demented individuals suffering from a persistent diarrhea of 15 to 25 stools a day for at least the past two months, and two cases with vicious dementia and mild diarrhea, weakness, and glossitis of seven and of thirty months duration   Although diarrhea was resistant to treatment, all cases recovered after a prolonged improved dietary regime   Skin lesions were observed in only one case and in this case the discoloration and fine desquamation over the feet were so greatly eclipsed by the violent dementia and diarrhea that he is included in this group   However, it is interesting to note that cystine in this case, 8 93 per cent, is the lowest value in the group   All values for cystine and total nitrogen (see Figure 1, D and Table I) fall within normal limits, cystine ranging from 8 93 to 11 98 per cent, and total nitrogen from 12 6 to 18 3 per cent   The average N S ratio approximates that of the normal group   Determinations after recovery in two cases show no striking change in cystine nor in total nitrogen

Group III consists of 10 cases all of whom had been recovering for the past two or three months from mild dermatitis of the feet and hands, and stomatitis   Intermittent diarrhea had been common and four cases had developed temporary psychoses   At the time of analyses dermatitis had disappeared and all were much improved   Cystine varied from 9 26 to 11 93 per cent, averaging 10 47 per cent, and total nitrogen 13 5 to 17 6 per cent with an average of 15 5 per cent, all within normal limits (see Figure 1, E and Table I)   The N S ratio averaged 5 56 with extreme of 4 57 and 6 16   Results from three cases following complete recovery showed no change except in one case in which cystine increased from 9 37 per cent to 12.25 per cent, total nitrogen from 15 4 per cent to 16 9 per cent and the N S ratio from 6 16 to 5 16

There are 21 cases of healed pellagra in Group IV   In this group analyses were made 3 to 6 months after disappearance of all signs of pel

lagra during which time the patient remained on an adequate diet containing an abundance of all vitamins Thirteen had recovered from severe pellagra with extensive dermatitis, four from severe pellagra of the gastrointestinal or central nervous system type in which dermatitis had been absent or played a minor role, and four from mild diarrhea and slight dermatitis In this group (see Figure 1 F and Table I) cystine varies from 8 22 to 15 42 per cent Two results 14 49 per cent and 15 42 per cent, lie above the upper normal limit Total nitrogen varies from 13 6 to 19 8 per cent also showing a greater variation than normal in the upper limit Both the average cystine and total nitrogen approximate the average of the normal group

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DISCUSSION AND SUMMARY

The foregoing results support the opinion that there is an abnormality of sulfur metabolism in pellagra which is reflected in epithelial tissue and specifically related to the dermatitis of this disease A study of the data from 36 normals and 20 cases of syphilis shows no constant variation of cystine with difference in sex race, or age Since cystine was reduced and N S ratio increased in the finger nails from all of the 14 cases of pellagra with severe dermatitis, whereas in all cases of pellagra without

dermatitis cystine and total nitrogen remained approximately normal it is reasonable to assume that the reduction of cystine in finger nail is closely related to the pathological changes in the epidermal tissues in pellagra This would seem to indicate that a lack of cystine or deficient utilization of this amino acid becomes manifest in the skin lesions of pellagra On the other hand, such a deficiency of cystine cannot be held responsible for the entire symptom complex of pellagra, since cases of pellagra without dermatitis in whom there is no diminution of cystine in the epidermal tissue, as indicated by our data, are common

Goldberger and Tanner (24) at one time advanced the theory of a deficiency of amino acids as the probable cause of pellagra and suggested that a deficiency in the supply or utilization of the amino acid, cystine, resulted in pellagra Support for this theory in explaining the skin lesions of pellagra is given by the work of Harris and of Ward previously cited and by our data Rough estimations of cystine in the dietary of our patients who developed pellagra failed to show a deficiency in the supply of cystine although a vitamin deficiency was probable The high cystine values in the finger nails in the group following dietary treatment may indicate greater cystine utilization influenced by adequate vitamin consumption However, as pointed out by Smith (25), the foods used in the treatment of pellagra contain large amounts of cystine and the increased concentration in healed cases may be due to increased intake of this amino acid per se, especially since Beadles et al (26) have demonstrated that the rat's coat of hair becomes heavier when the diet is supplemented with cystine

Injections of thiosulfate have been advocated and extensively used with apparent benefit in the treatment of pellagra Sabry (27) reports that the skin lesions of pellagra disappear after a few injections, and that there is marked improvement of diarrhea and of the central nervous system manifestations of this disease after a short course of treatment, consisting of daily intravenous 10 cc injections of 10 per cent solution of sodium thiosulfate Although Goldberger et al (19, 20) have demonstrated that pellagra may be prevented and cured by supplying the pellagra-preventing factor (vitamin G) in the diet of the pellagrin, and although the thiosulfate treatment is usually combined with an improved dietary regime, in view of the demonstrated alteration in sulfur metabolism, it is likely that thiosulfate is of some benefit to the pellagrin and that some of the rapid improvements observed may be due to this factor directly

Although we cannot conclude that because of a reduction of cystine in finger nails there must be a reduction of cystine in the skin and therefore an increased sensitiveness to light, it is reasonable to suspect that a reduction of nail cystine is accompanied by a reduction of skin cystine We contemplate further experimental work to determine the importance of this change in sulfur metabolism and its relation to solar radiation in pellagra

CONCLUSIONS

1 The cystine content of finger nails was determined in 36 normal subjects in 40 cases of various forms and stages of pellagra and in 25 cases of syphilis and tuberculosis

2 In 14 cases of pellagra with extensive dermatitis there was a marked reduction in the cystine content without any appreciable change in the total protein content of the finger nails   With the subsidence of skin symptoms and improvement in the clinical condition the cystine content returned to normal limits

3 In severe pellagrins without dermatitis (involvement chiefly of the gastro intestinal or central nervous systems) and in partially or completely cured cases of pellagra there were no marked changes in the cystine content of the finger nails

4 Values for cystine content of the finger nails from a group of syphilitics and from a group of tuberculous patients were found to approximate the normal range regardless of the nutritional state of the patient

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THE PRODUCTION OF EXPERIMENTAL PLAUT–VINCENT'S ANGINA IN THE DOG

By HELENE WALLACE, E. W. WALLACE and O. H. ROBERTSON

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Since the original description of Plaut-Vincent's angina some fifty years ago a voluminous literature on this subject has accumulated. It is not the purpose of this paper to review the literature in any detail as this has been done elsewhere (1, 2). However it may be stated briefly that the disease was described in 1883 by Miller (18), in 1894 by Plaut (3) and in 1896 by Vincent (4) as manifested by sore and bleeding gums, foul breath and in severe cases by eventual ulceration of the mouth. They described the causative micro organisms as a spirochete and a fusiform bacillus. Since then these organisms have been found associated with pathological lesions in practically every part of the body but especially in the upper digestive system, the lungs and the genitalia.

Upon the isolation of the etiological agents the question was immediately raised as to whether they were separate entities or different forms of the same micro organism. Weaver and Tunnicliff (5) succeeded early in cultivating the fusiform bacillus from a patient with Vincent's angina and on the basis of further work Tunnicliff (6) has stated that she believes the spirochete and bacillus are the same organism in different stages of a definite life cycle. Some workers (7) have supported her but others (8) and in particular Pratt (9) have come to the conclusion that these are two distinct micro organisms.

The early literature stressed the infectious nature of the disease but more recent observations indicate that the causative organisms are found in the normal mouth rather constantly and that the inception of infection depends on the presence of certain predisposing factors. Pratt found fusiform bacilli in 100 per cent of the two hundred people routinely examined at a large hospital. She also found the fusiform bacillus constantly and the spirochete occasionally in normal monkeys, guinea pigs and rabbits. Other workers have reported analogous findings in these and other animals (2). Thus it would seem that the fusiform bacillus at least is present in the healthy mouth and is not in itself pathogenic. This is borne out by our work. Of forty dogs examined thirty eight showed the bacillus and in those with poor teeth, spirochetes were usually present in the teeth pockets.[1]

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[1] Vincent's angina is a recognized disease of the dog's mouth.

Many attempts have been made to reproduce the disease experimentally. In 1923 Kline (10) produced abscesses in the traumatized groin of a guinea pig by injecting membranes from cases of Vincent's angina. Somewhat later Smith (11) succeeded in producing similar abscesses in the untraumatized guinea pig groin by injection of material freshly obtained from human cases. He then injected into the lungs of rabbits pus from these experimental abscesses which showed spirochetes and fusiform bacilli in addition to other microorganisms. Of ten rabbits injected four developed lung abscesses, three gangrene, two pneumonia and one bronchiectasis. From certain of these lesions he isolated spirochetes and fusiform bacilli. Randall (12) injected scrapings from the gums of patients suffering from Vincent's angina into white rats and Warren (13) into kittens without success. Both workers used animals fed on a deficiency diet as well as normal ones. Wagener (13) traumatized the gums severely in some instances and made deep artificial pockets into which she injected the material but the wounds healed and no fusiform bacilli or spirochetes could be recovered. Lichtenberg, Werner and Lueck (14) were unable to produce lesions in guinea pigs by the injection of pure cultures of fusiform bacilli into traumatized tissues but did find spirochetes and fusiform bacilli in the membranes which formed over ulcers induced by injury of the mouth in normal guinea pigs.

While the above data indicate that experimental lesions of the groin and lung have been induced by the implantation of spirochetes and fusiform bacilli the reproduction in animals of the clinical symptoms of Vincent's angina has not been successful.

In the spring of 1931 one of the authors (15) observed that a group of the dogs which he had been injecting daily with one of the squill gluco-sides namely, scillaren B developed a peculiar mouth condition similar to that found in Plaut-Vincent's disease. Smears from the mouths of these dogs showed the typical fusiform bacilli and spirochetes in large numbers. This observation suggested a possible method for the experimental production of Vincent's angina.

<center>METHODS AND MATERIALS</center>

mal was injected daily intravenously   The dogs injected with scillaren B were not run in a single series but one or two at a time simultaneously with other animals receiving different squill glucosides   A total of forty eight dogs were used   Each drug was tested on eight dogs   Conditions of care, hygiene and type of food were identical for all the dogs

The dogs used in the present experiments were selected primarily for their good teeth and gums   they were all lively and in excellent condition   For the ease of handling the small terrier type was chosen   Temperature and pulse were taken twice daily   weight and (in the case of five dogs) a white count once a day   Detailed records by means of sketches and notes were made daily of the teeth gums and mouth of each animal   At the same time smears were taken from each side of the mouth the incisors and any suspicious pockets   The smears were stained by Tunicliff s method [3]   All the animals were kept under excellent conditions and were well fed   The food given contained ade quate amounts of the various vitamins

A saline solution of the drug was injected daily into the antebrachial vein of the dog s foreleg   About 0 30 of the lethal dose was given for the first three days and subsequently 0 25 of the lethal dose until death   (The lethal dose as measured by the Hatcher Brody cat method is 0 1 mgm per kilo of body weight)   Photographs were taken of the dogs mouths before the injection of the drug was begun in various stages of the disease and in two cases five minutes after death

Autopsies were performed immediately after death   Particular attention was paid to the mouth esophagus stomach trachea lungs heart and kidneys Tissues from ulcerating areas as well as normal and only slightly affected re gions were excised and sectioned   These tissues were usually fixed in Zenker s acetic (in the case of the silver methods for spirochetes 10 per cent neutral formalin was also used)   The sections were for the most part stained with Giemsa s stain prepared by Grubler as this was found to stain both the spiro chetes and fusiform bacilli distinctly and also to give the best histological pic ture   Levaditi s silver method for spirochetes was also of value

During the course of the disease in the animals numerous cultures from the mouth were made

EXPERIMENTAL

In the present study eleven dogs six males and five females were in jected with scillaren B   All of them developed the characteristic lesions and bacteriological findings of Vincent s angina   In three dogs (4 5 and 8 Table I) the lesions were minimal consisting of redness and sensitive ness of the gums with some bleeding discoloration of the teeth and the presence of numerous fusiform bacilli and some spirochetes   Two of these three animals 5 and 8 both of them very young dogs, died of an acute respiratory infection at this stage [4]   Dogs 1 3 10 and 11 exhibited a well marked clinical picture of the disease with one or two ulcerations   Dogs 2 6 7 and 9 developed a severe form of the infection   In all these eight

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[3] The stain made up of 90 cc of a saturated 95 per cent alcoholic solution of Grubler s gentian violet added to 10 cc of 5 per cent phenol was poured over the dried smears and steamed for five minutes   The slides were then washed with tap water blotted with filter paper and examined under oil

[4] Not fuso spirochetal as far as could be determined

TABI I
Summary of data

Dog num ber	Sex	Weight		Temperature				Pulse		White blood cells	Number of days injected*	Severity of disease	Additional data
		1st day	At death	1st day	Aver age	Day of death	Average pulse before injection	Day and rate of slowest pulse		*thousands*			
1	M	116	99	101	101	101	111	4th—60	10, 24, 14	7 D	Severe	Different sullivan B Powder used More potent?
2	M	11 25	69	102 3	102	99	116	5th—48	7 5, 9, 35	11 D	Very Severe	
3	M	9	51	101	101	100	110	12th—56	5 12 5, 7	16 D	Very Severe	Died of respiratory infection
4	M	71	5	101	101 2	99 6	100	28th—28	14, 16, 16	28	Very Severe	Died of respiratory infection
5	M	11 3	50	102	101	101	116	10th—70	8, 20	12 D	Moderate	Drug discontinued (see text)
6	M	7	50	102	101	96 6	116	5th—52		15 D	Very Severe	Animal recovered
7	M	1	71	102	100	98 5	124	6th—40		12 D	Very Severe	
8	M	9	92	102	101	105	110	3d—55		5 D	Moderate	Died of respiratory infection
9	M	9 5	65	101 5	101	99 5	110	5th—48		15	Severe	Animal allowed to recover
10	M	1 5	101	100 5	100 6	100	120	9th—42		14 D	Severe	Injected 2d and 3d times Subsequent re
11	M	12	8 1	101 5	101	100	116	7th—52		11 D	Severe	tubule severe

* Dogs injected until death with the exception of No. 4 and No. 9
D Died

animals the molars became black and the canines a very dark brown   Ulceration of various parts of the cheeks and tongue occurred   These ulcers were usually one to two centimeters in diameter   Several were considerably larger   There was much bleeding from the lesions

As the disease progressed in a similar fashion in all the dogs a general description may be made   During the first two days the dogs were apparently normal but after the injection on the third day, they all vomited profusely and were somewhat depressed   By this time smears from the mouth usually showed a decided increase in the number of small, short fusiform bacilli   By the fourth or fifth day vomiting was acute and very little food was eaten   The teeth generally the posterior molars and canines were somewhat discolored the gum margins showed a narrow line of inflammation and the smears from these regions contained quantities of short fusiform bacilli and a sprinkling of spirochetes   By the sixth day the dogs were no longer lively or responsive although the vomiting was not as acute   The gums were markedly inflamed and bleeding, the teeth darker, and the smears showed larger and more typical cigar shaped bacilli and also spirochetes   From the seventh to the fourteenth day the teeth became progressively darker until all but the incisors were a dark brown the gums bled profusely and were very friable and ulcers formed on the gums cheek and tongue (Fig 1)   The smears showed large typical fusiform bacilli and spirochetes   Quantities of the latter were found wherever there was a bleeding and necrotic pocket

During the injections the dogs lost a great deal of weight in some cases almost half the original body weight   There was no fever but rather a drop in temperature during the last few days of the disease

The white blood cell counts made on the first three dogs showed a decided increase at first but one or two days before death the leukocytes dropped considerably though not always below what might be considered normal   In Dog 4 the white count did not go down but remained at a rather high level   This animal exhibited the mildest lesions in the series   Dog 5 which died of a concurrent respiratory infection showed a rise in the white blood cells to 20 000   However the leukocytosis apparently did not hinder the development of the Vincent's angina

The animals died on an average about the fourteenth day in ventricular fibrillation   However one dog died as early as the seventh day and another (Dog 4) did not succumb to twenty eight injections   Most of the dogs were injected until they succumbed from the cumulative effects of the drug   In two animals the drug was discontinued as shown in Table I (Dogs 4 and 9)   Dog 9 recovered from a severe attack of the experimental disease and two months later when a smear from the mouth showed only the few fusiform bacilli usually found in normal dogs he was again injected and developed a pronounced Vincent's angina as before   After a second recovery and a three months interval a third attack was induced   The second and third attacks were not as severe as the first

used for other experimental purposes [5] Dogs 5 and 8, the two animals
that died of a respiratory infection had a final temperature of 104 and
105° F respectively However early in the course of the disease their
temperatures were normal

As shown in the table the average pulse rate for three days before in
jection was about 112 After the injection of the drug an initial progres
sive slowing of the heart occurred Electrocardiograms taken at the time
when the rate was slowest showed the heart to be in a condition of partial
to complete block Apparently the drug in this phase of its action pro
gressively lengthened the conduction time until there was complete dis
sociation between auricular and ventricular contractions The day on
which the pulse was slowest generally occurred about the middle of the
injection period Later as more toxic amounts of the drug accumulated
the heart rate increased Apparently the ventricular musculature itself
was stimulated Ectopic foci appeared at various points in the ventricle,
producing irregular QRS-T complexes of high potential and bizarre shapes
Before death the heart rate was usually too rapid to be counted

Attempted transmission
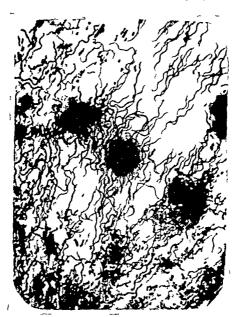
Several control animals were kept in the room with the injected dogs
and although they were allowed to eat out of the same pan as dogs with
well advanced cases of Vincent s angina they did not contract the disease
Swabs were then taken from the necrotic pockets in the gums of Dogs 6
and 7 and pushed into the gum margins of the control animals without any
apparent effect save that in one dog, whose physical condition was some-
what poorer than the others there was some transient local redness and
tenderness of the gums

Pathology

At autopsy lesions were found only in the mouth All the internal
organs appeared normal An extremely foul odor came from the mouth
when it was opened The teeth were badly discolored the canines and
molars being the most affected Necrotic pockets filled with spirochetes
were usually present at the base of these discolored teeth In only one
animal (Dog 3) were the incisors affected In this instance the molars
were less involved than the incisors and the single ulcer present was at the
base of the lower incisors In Dogs 2 6 7 and 9 there were extensive
cheek ulcers on both sides of the mouth The first three of these dogs
also had from one to five ulcers on the tongue one to two centimeters long
and one half to one centimeter wide The largest ulcers were those on the
cheek of Dog 7 where the whole right side of the mouth was involved
All the ulcers although irregular in shape had well defined margins and

fusiform bacilli only became numerous about the sixth day at which time spirochetes and necrosis also appeared The spirochetes were found in abundance in the pockets and necrotic regions (Fig 2) The typical'

FIG 2   Dog No 9

Smear taken from necrotic pocket in gum on tenth day of disease shows masses of spirochetes   1200 ×

Vincent s angina smear (Fig 3) could be obtained at the end of the first week of injection and at any subsequent time In this microphotograph the spore like character of the fusiform bacilli may be seen Long filaments of fusiform bacilli were not uncommonly observed (Fig 4) In some cases they resembled the filamentous forms seen in the cultures and sections The smears at this time showed practically no other organisms than the fusiform bacilli and spirochetes

A study of a number of human cases of Vincent s angina gave analogous findings They showed all the variations of the fusiform bacilli that were present in the dogs mouths Likewise in the human cases the spirochetes were found in the teeth pockets or deep in the necrotic tissue such as occurred in tonsillar crypts

Cultures were taken from the dogs' mouths when the smears showed many spirochetes and fusiform bacilli. A number of cultures were also made from the pockets about the teeth, ulcers, and regions where the smears showed a predominance of one micro organism or the other. Most of the cultures were made with material from Dog 9.

Various culture media were used. The fusiform bacilli grew fairly well on rabbit's blood agar (one part of the blood to three parts of beef infusion agar, pH 7.4 to 7.6) but not nearly as readily as on ascitic agar (one part of ascitic fluid to three parts of a beef infusion agar, pH 7.4 to 7.6). Ascitic agar and a beef infusion—ascitic broth (three parts of one per cent dextrose beef infusion broth to one part ascitic fluid, pH 7.4 to 7.6) were our most satisfactory media. With Loeffler's medium recommended by Tunnicliff, we were less successful. We also used Noguchi's leptospira medium (17) in an attempt to grow the spirochetes.

All the cultures were grown anaerobically. Although we tried anaerobic plates as well as anaerobic jars in which the air had been evacuated and replaced with CO or nitrogen we found that Wright's anaerobic method besides being the simplest was also the best for our purpose. Test tubes were stoppered by cotton saturated with a five per cent NaOH solution. A small amount of pyrogallic acid was placed on top of this and the tube corked and sealed with paraffin. Incubating temperature was 37° C.

We were able to isolate colonies of the fusiform bacilli quite readily after forty eight hours incubation. They were minute and while not absolutely transparent, they seemed so when contrasted with the opaque colonies of cocci which formed the bulk of the growth. Single colonies were transferred to ascitic agar slants and were maintained in pure culture without difficulty. Transplants to fresh media were made every three days for two weeks and subsequently every two weeks for three months. After some months the bacillary forms became more or less degenerate although a slant examined eleven months subsequent to the initial culture still contained some recognizable fusiform bacilli. At no time were we able to cultivate the spirochetes—even when the inoculum was obtained from pockets showing practically a pure growth of spirochetes nor did we find spirochetes at any time in our cultures of fusiform bacilli. Subcultures of fusiform bacilli in leptospira medium yielded only fusiform bacilli.

The fusiform bacilli in the pure cultures were small at first but grew rapidly to the usual size and subsequently into the long filamentous forms which at times stretched completely across the microscopic field. They were also often wavy and to the casual observer might be taken for spirochetes. They were however much larger than the Vincent's spirochete and under dark field examination were seen to be non motile.

As a rule once the fusiform bacilli had attained the filamentous stage they showed no further change. Occasionally small fusiform bacilli appeared in cultures in which only filamentous forms had been observed for

that the injection of scillaren B disturbs metabolic processes in such a manner as to lower the resistance of the buccal mucous membrane to the spirochetes and fusiform bacilli normally present   Whether this deleterious effect of the drug is principally a generalized or a local one we do not know   While it is true that the animals all showed marked gastro intestinal disturbances and an associated loss in weight these same effects were produced to an equal degree by the other squill derivatives employed but without the subsequent development of fuso spirochetal mouth lesions except in one instance   Thus it would seem that either the general effect of scillaren B is of a peculiar nature or that it produces a characteristic local disturbance not shown by the five other closely related compounds   The excretion of the drug locally in the mouth cannot be ruled out as a cause of the lesion but neither can it be determined as such since the amounts injected are far too small to render recovery of the drug possible   It is of course conceivable that the production of the lesions in the mouth by this one squill glucoside is due to impurities in the preparation rather than to the compound itself   Of two lots of scillaren B used one was found to cause the development of the experimental disease much more quickly than the other

The data presented indicate that a leukopenia is not a factor in favoring inception of the disease nor does a leukocytosis appear to influence its course

Our unsuccessful attempts to transmit the disease to healthy dogs while too few in number to permit conclusions suggest that the fusiform bacilli and spirochetes may not be pathogenic for the normal animal even when applied to the gums and mucous membranes of the mouth in large numbers   In a recent paper, Lichtenberg and his co workers (14) question the pathogenicity of these micro organisms for normal human beings   However there are well authenticated reports in the literature of small epidemics of Vincent s angina among normal persons living under apparently healthful conditions   This leads one to infer that either the normal dog possesses a greater natural resistance to the fuso spirochetal micro organisms than do human beings or that the strain found commonly in the dog differs in pathogenicity from that occurring in the human mouth

With the evidence available we feel that it is not yet possible to draw definite conclusions concerning the identity or non identity of the spirochete and fusiform bacillus   Our cultural studies do suggest that these are two distinct microbial forms   If the fusiform bacillus and the spirochete represented different phases of a single life cycle one might expect that in a culture medium highly favorable to the multiplication of the bacillary form the spirochetal phase would develop   However since we have not been able to contrive sufficiently favorable conditions in the test tube for the growth of spirochetes freshly isolated from the lesions we have no means of knowing whether or not spirochetes would evolve from fusiform bacilli were a suitable growth medium provided

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THE EFFECTS OF ALTERNATE SUCTION AND PRESSURE ON BLOOD FLOW TO THE LOWER EXTREMITIES

By EUGENE M LANDIS and JOHN H GIBBON, JR.

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From the therapeutic standpoint patients with peripheral vascular disease are divided into two groups, (*a*) those whose symptoms are due to simple spasm with no, or very slight, organic vascular obstruction and (*b*) those whose symptoms are due primarily to advanced organic disease of the arteries   The symptoms of the first group can usually be alleviated by producing vasodilatation with drugs diathermy, local heat in the form of the warm cradle, or sympathetic ganglionectomy   The efficacy of these therapeutic procedures depends upon local dilatation of the peripheral blood vessels

In more advanced stages, however, obliterative structural disease has usually progressed until the arterial vessels are transformed into rigid tubes   These diseased vessels have not only abnormally small lumina but are also more or less incapable of dilatation even when vasomotor tone is reduced by nerve block or by sympathetic ganglionectomy   When the arteries have thus lost their power of dilating the therapeutic procedures mentioned above fail to increase blood flow and therefore fail to improve the nutrition of the tissues   Eventually many of these patients with advanced organic disease of the arteries suffer from trophic changes, ulceration and gangrene leading finally to amputation   Any procedure which tends to increase blood flow in the presence of organic occlusion might be of benefit in delaying or possibly in preventing the appearance of gangrene

According to Poiseuille's law the amount of fluid flowing through a rigid tube depends upon the fall in pressure along the tube   If other factors remain constant doubling the peripheral fall in blood pressure should approximately double the amount of blood flowing through the rigid arterial vessels   The total fall in pressure in the peripheral vascular system may be increased in two ways   (*a*) by elevating systemic blood pressure or (*b*) by diminishing capillary and venous blood pressures   The first method is impracticable for numerous reasons   It seemed possible, however, that capillary and venous blood pressures might be reduced temporarily to negative values (i e , below atmospheric pressure) by applying suction to the skin of the extremity

The full effect of externally applied negative pressure can only be obtained if there be a reservoir capable of accommodating the blood which

has flowed through the narrowed arteries. The capillaries and veins which are not constricted offer such a reservoir—of limited capacity. Landis and Gibbon (1) observed that inflating a pneumatic cuff on the upper arm to a pressure of 30 cm. water for 5 minutes increased the volume of a segment of forearm by 25 cc. of which 3 cc. consisted of extravascular fluid while 22 cc. consisted of blood trapped in the congested peripheral vessels. Similarly, a venous pressure of 60 cm. water during 6 minutes increased the volume by 38 cc. of which 7 cc. was extravascular fluid and 31 cc. was blood in the congested vessels. The total volume of the segment of forearm was 720 cc. so that at pressures of 30 and 60 cm. water the reservoir available was 3 and 4 cc. per 100 cc. of arm respectively. Average blood flow in the normal forearm amounts usually to less than 5 cc. per minute per 100 cc. of tissue (Lewis and Grant (2)). Hence, with a slight rise of pressure the veins and capillaries can accommodate the blood flowing through normal vessels during a period of approximately one minute.

From these observations it seemed possible that the periodic application of negative pressure to the surface of the lower extremity might be expected to increase blood flow even though the arterioles had been changed into rigid tubes by reason of structural disease. Moreover, if the suction were applied for relatively short periods of time the efficiency of the method would be greater since the available reservoir would not be filled to capacity during any one suction period. The accumulation of blood in the capillaries and veins must lead to stretching of their walls, the resistance to further distention increasing as the amount of trapped blood became greater. This resistance would of course, lessen the effect of external suction on the peripheral drop in blood pressure particularly if suction were prolonged. It seemed essential, therefore, not only to use relatively brief periods of suction but also to empty the capillary and venous beds of their contained blood after each brief suction period, so that space might be available for the accommodation of fresh arterial blood to be drawn in during the succeeding suction period. Therefore, suction and pressure were applied alternately.

The effects of alternate suction and pressure on flow have been studied in a circulatory schema and in the lower extremities of normal subjects and of patients with peripheral vascular disease. This paper provides objective evidence that blood flow can be increased by this means. The clinical value of this procedure will be considered in later publications. It seemed advisable first to demonstrate objectively and under controlled conditions, the correctness of the working hypothesis.

APPARATUS AND METHOD

was 28 cm wide 75 cm long 28 cm high at the proximal end and 34 cm high at the distal end   These dimensions permitted the lower extremity to be inserted to a point approximately 8 inches above the knee joint (Fig 1)   The upper
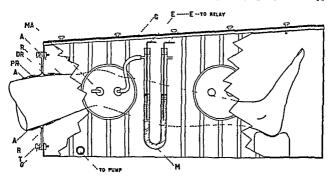
FIG 1   DIAGRAM OF ALUMINIUM BOX USED TO APPLY SUCTION AND
PRESSURE TO THE LOWER EXTREMITY

For description see text

surface of the box could be removed and consisted of a sheet of shatter proof glass (G) mounted in a holder which was screwed down upon a rubber gasket. The foot and leg could thus be observed during the application of suction and pressure   The proximal end of the box contained a circular opening (O) measuring 25 cm in diameter which was fitted with a rubber cuff screwed tightly against the end of the box by means of thumb screws (T)   The cuff consisted of a semi rigid ring (R) of rubber and canvas with an external diameter of 28 cm and an internal diameter of 14 cm   The internal portion of the cuff consisted of two leaves of lighter flexible rubber having a thickness of 1 5 to 2 mm   These leaves were conical in shape, the smaller end of one (PR) being directed toward the hip while the smaller end of the other (DR) was directed toward the knee   The dimensions of the cuff were adjusted for different subjects so that the flexible rubber fitted snugly against the skin of the thigh   The fit was made as accurate as possible in order to avoid both excessive venous congestion and excessive leakage of air   The rubber was held lightly in contact with the skin by adhesive tape (A)   The heel rested on a sand bag and for greater comfort the lower thigh was supported by sand bags placed just within the rubber diaphragm

The interior of the box communicated through three-quarter inch rubber tubing with an air pump of the Crowell type (size 2A) having a capacity of 13 liters per minute at atmospheric pressure   The interior of the box also communicated with a mercury manometer (M) made of glass tubing with an internal diameter of 1 cm   A metal electrode (E) was inserted into each limb of the manometer   These electrodes were connected with a difference of potential and with the coil of an electrical relay   The contacts of the relay operated a one half horse power A C (Westinghouse Type RV) motor connected by a leather belt to the air pump   A motor driven 3 way metal valve placed the box in communication with the intake pipe of the pump for 25 seconds, and with the

output pipe of the pump for 5 seconds. The manometer and valve therefore controlled automatically (by the positions of the two electrodes in the manometer) the amount of suction or pressure applied, while the valve controlled the duration of the suction and pressure periods.

In all but two of the observations here reported a negative pressure of 120 mm Hg was applied for periods of 25 seconds, alternating with a positive pressure of 80 to 100 mm Hg for periods of 5 seconds. It required approximately 3 to 5 seconds to change the pressure within the box from —120 to ₊120 mm Hg. For observing the final pressures more accurately a second manometer communicated with the interior of the box through a separate opening (M4).

The air expelled by the pump warmed in the process of compression. Since changes in blood flow were detected by recording skin temperature it was necessary to cool the air entering the box during the pressure periods. The air was therefore passed through a honeycomb radiator immersed in water at 6 to 10° C. This prevented excessive warming of the air surrounding the lower extremity.

Changes in cutaneous blood flow in the lower extremity were measured qualitatively by observing skin temperature. Three copper-constantan junction passed into the interior of the box through a rubber stopper inserted in the side wall. One junction was fixed to the skin at the base of the nail of the first toe, another junction was similarly situated on the skin of the third toe. Each junction was covered with one layer of adhesive plaster. The third thermal junction was suspended near the foot to record the temperature of the air around the extremity.

Since in these observations increase in blood flow was identified by elevation of skin temperature it was essential to control the observations very carefully. Therefore, while one lower extremity was placed in the aluminium box the other lower extremity was used as a control and inserted into a second box having the same dimensions and the same general shape as the aluminium box. This control box was fitted with one 10-watt and one 50-watt lamp, which could be turned on separately or together in order to keep the temperature of the air in the control box always above that of the air in the aluminium box.

Conditions were arranged to favor greater cooling of the extremity exposed to suction and pressure. First, the air temperature in the control box was higher than that in the aluminium box. Second, the air in the control box was still while that in the aluminium box was moving due to alternate removal and introduction of air during suction and pressure respectively. Third, the extremity in the aluminium box was slightly congested by the rubber cuff while the extremity in the control box was not congested; the space between the skin of the thigh and the proximal end of the control box being closed loosely with cotton. The conditions were thus arranged to favor cooling of the experimental extremity as compared to the control extremity, so that if the extremity exposed to suction and pressure became warmer than the control extremity, the change might be safely ascribed to increased blood flow.

OBSERVATIONS

fluid through a partially obstructed rigid tube   In the schema various lengths of capillary glass tube simulated an arterial obstruction while a rubber bag simulated the distensible capillary and venous beds   A valve was inserted in the outflow (or venous) tube leading from the bag to simulate the venous valves

The rate at which water flowed through the system under a pressure of 80 mm Hg (109 cm water) was first measured over several periods of six minutes without pressure variations, i e , with atmospheric pressure both inside and outside the box   The first column in Table 1 shows the single and average measurements of the outflow in cubic centimeters per minute   Suction (— 120 mm Hg) was then repeatedly applied to the portions of the schema within the box for varying periods—25, 55, 85, and 115 seconds—with alternate periods of pressure

Flow was uniformly greater during suction and pressure   The percentage increase in flow is recorded in the right half of Table 1   Suction for 25 seconds and pressure for 5 seconds increased the outflow by 45 to 63 per cent   With longer periods of suction slightly greater increments in flow were observed since the total drop in pressure was increased for a slightly greater proportion of time, relatively less time being wasted in changing from negative to positive pressure

B   Observations on normal subjects

1   The effects of suction and pressure on the rate of cooling of the previously warm lower extremity

At low room temperatures the extremities of normal subjects cool rapidly as a result of peripheral vasoconstriction which serves to conserve heat by diminishing radiation   As shown by Gibbon and Landis (3) when the forearms are immersed in warm water maximal vasodilatation is produced in the lower extremities, and the surface temperature of the digits rises to 32° C or more   In this series of observations the subjects were exposed to low room temperatures and maximal vasodilatation was first produced in the lower extremities by immersing the forearms in warm water   The rate at which the lower extremities cooled following such maximal vasodilatation was observed both with and without pressure changes in order to ascertain whether alternate suction and pressure modified the rate of cooling

The subjects were seated on an examining table with the legs extended horizontally while the back rested against a support at an angle of 45 to 60°   The right lower extremity was placed in the control box, the air in which was kept slightly warmer than that of the aluminium box   The left lower extremity was inserted into the aluminium box through the rubber cuff

Figure 2A shows the characteristic result obtained when the two extremities were first warmed through peripheral vasodilatation and then

TABLE 1

Effects of alternate suction and pressure on the flow of water through a circulation schema

	Outflow from venous end of schema						Percentage increase in outflow produced by suction and pressure				
		With suction and pressure									
Without suction and pressure	Suction 25 seconds Pressure 5 seconds	Suction 55 seconds Pressure 5 seconds	Suction 85 seconds Pressure 5 seconds	Suction 115 seconds Pressure 5 seconds			Suction 25 seconds Pressure 5 seconds	Suction 55 seconds Pressure 5 seconds	Suction 85 seconds Pressure 5 seconds	Suction 115 seconds Pressure 5 seconds
cc per minute 150	cc per minute 217	cc per minute 212.5	cc per minute 211	cc per minute 251.5			per cent 45	per cent	per cent 61	per cent 78
152 113 112 110 111 111 110 } 113	218 222 } 232.5	210 216 } 212.5	252 252 } 252	250 259 } 251.5			63	70	76	
50 59 58 48 } 49	75 75 74 76	67 70 77 77 } 75	78 75 70 79 } 79				58	58	61	
47.5 47.5 47.5 } 47.5	76 77 74 75	69 76 79 79 } 76	70 79 79 79 } 77					60	62	

FIG 2*A*   CONTROL OBSERVATION SHOWING COOLING OF THE DIGITS OF THE
LOWER EXTREMITIES WITHOUT SUCTION AND PRESSURE

In this and subsequent charts R1 indicates the skin temperature of the right
first digit, L1 the skin temperature of the left first digit
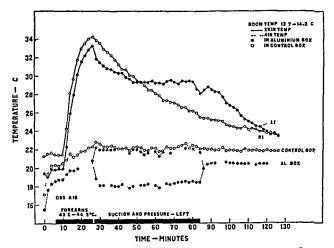
FIG 2*B*   SHOWING COOLING OF THE DIGITS DURING APPLICATION OF SUCTION
AND PRESSURE TO THE LEFT LOWER EXTREMITY

45

allowed to cool in their respective boxes without suction and pressure being applied to the left lower extremity. As was to be expected on account of the warmer air in the control box, the temperature received during vasodilatation was higher in the right extremity. After the forearms were removed from the warm baths vasoconstrictor tone increased and the right and left extremities cooled at approximately the same rate, the right extremity remaining throughout slightly warmer than the left. The subjects were chilly or shivering while the lower extremities cooled.

Six such control observations are summarized in Table 2. Room temperature ranged from 10.7 to 16.3° C. At the beginning of the cooling period the surface temperatures of the digits were all above 33° C, the right digits being warmer than the left. The duration of the cooling period varied from 38 to 58 minutes and in this time the temperatures of all the digits became lower. With one minor exception the left digits remained as cool as, or cooler than, the corresponding right digits throughout the cooling period. The temperatures of the air in the aluminium and control boxes were read every two minutes. The figures recorded in Table 2 as "average temperature of air in boxes" at the beginning and end of the cooling periods represent respectively the average of the five readings made during the first 10 minutes and the last 10 minutes of the cooling period. It may be observed that the air in the control box was warmer than the air in the aluminium box by 1.1 to 1.7° C.

The relation between the surface temperatures of the right and left digits is summarized in the last two columns of Table 2. The temperature of the right first digit at the beginning of the cooling period has been subtracted from that of the left first digit observed at the same time. The left and right third digits have been similarly compared as regards temperature. The minus sign indicates that the left digit was the cooler by the amount given. At the beginning of the cooling period the left digits were from 0.1 to 2.5° C cooler than the right. At the end of the cooling period the same relation existed in exaggerated form, the left digits being, with one exception, from 0.0 to 3.8° C cooler than the right. The single exception occurred in Observation 16 in which the left third digit became 0.3° C warmer than the right third digit. The figures given in the lower right hand corner of Table 2 indicate that the left digits averaged 0.8° C cooler than the right at the beginning of the cooling period and 1.8° C cooler at the end of the period. These control observations show that under the environmental conditions mentioned the left digits remained definitely cooler than the right when suction and pressure were not applied to the left extremity.

TABLE 2

Cooling of feet without suction and pressure

Observation	Subject	Room temperature	Digit	Skin temperature		Duration of cooling period	Average temperature of air in boxes		Difference in skin temperature {L1 R1 / L3 R3}	
				At beginning of cooling period	At end of cooling period		Beginning of cooling period	End of cooling period	Begin ning of cooling period	End of cooling period
		C		C	C	minutes	C	C	C	C
A16	G	13 7–15 0	L1	33 6	26 8		22 5	22 0	−1 1	−1 4
			L3	33 1	25 8	40			−0 5	+0 3
			R1	34 7	28 2		23 9	23 1		
			R3	33 6	25 5					
A26	G	11 0–13 8	L1	33 4	28 6		22 9	22 3	−2 5	−2 9
			L3	33 4	26 0	50			−1 2	0 0
			R1	35 9	31 5		24 5	23 7		
			R3	34 6	26 0					
A28	G	13 0–13 5	L1	34 0	25 3		20 9	20 1	−1 0	−1 6
			L3	33 3	23 6	38			−0 2	−0 4
			R1	35 0	26 9		22 5	21 6		
			R3	33 5	24 0					
A29	L	14 0–15 2	L1	35 9	32 9		23 6	22 4	−0 2	−1 1
			L3	34 6	30 5	50			−1 2	−2 1
			R1	36 1	34 0		25 3	24 0		
			R3	35 8	32 6					
A30	L	15 5–16 3	L1	34 7	29 3		21 9	21 6	−0 1	−2 5
			L3	33 8	28 0	58			−0 3	−2 4
			R1	34 8	31 8		23 5	23 1		
			R3	34 1	30 4					
A31	L	10 7–13 0	L1	33 4	27 3		20 5	19 8	−0 3	−3 8
			L3	33 7	26 9	56			−0 9	−3 6
			R1	33 7	31 1		22 1	21 5		
			R3	34 6	30 5					
								Average	−0 8	−1 8

The air in the aluminum box became warmer during compression and cooler during rarefaction as was to be expected from physical principles The range of these fluctuations during suction and pressure has been shown in Figure 2B (et seq) by dividing the line showing air temperature in the aluminium box into two lines, of which the upper represents the

allowed to cool in their respective boxes without suction and pressure being applied to the left lower extremity. As was to be expected, on account of the warmer air in the control box, the temperature reached during vasodilatation was higher in the right extremity. After the forearms were removed from the warm baths vasoconstrictor tone increased and the right and left extremities cooled at approximately the same rate, the right extremity remaining throughout slightly warmer than the left. The subjects were chilly or shivering while the lower extremities cooled.

TABLE 3—(Continued)

Observation number	Subject	Room temperature	Digit	Skin temperature			Duration of suction and pressure (left only)	Duration of period without pressure changes	Average temperature of air in boxes			Difference in skin temperature {L1 R1 / L3 R3}		
				Beginning of period of suction and pressure	End of period of suction and pressure	End of period without pressure changes			Begnning of pressure changes	End of pressure changes	End of period without pressure changes	Begin ning of pressure changes	End of pressure changes	End of period without pressure changes
		C		C	C	C	minutes	minutes	C	C	C	C	C	C
A19	L	15 0–15 8	L1	33 6	31 2	27 8	59	36	21 5	21 9	22 4	−1 3	+3 2	+0 4
			L3	34 0	29 4	27 5						−0 5	+1 9	+0 5
			R1	34 9	28 0	27 4	None		23 3	23 6	23 3			
			R3	34 5	27 5	27 0								
A22	G	12 8–13 2	L1	34 2	30 5	26 8	50	30	21 9	22 7	21 8	−0 1	+1 8	+0 3
			L3	32 2	25 9	24 7						−1 6	−0 3	+0 2
			R1	34 3	28 7	26 5	None		24 1	24 8	24 9			
			R3	33 8	26 2	24 5								
A6*	L	14 2–15 8	L1	33 6	32 9	28 9	66	46	17 6	18 7	19 5	−0 6	+2 6	−1 1
			L3	31 6	29 3	26 1						+0 1	+1 4	−0 8
			R1	34 2	30 3	30 0	None		20 2	21 8	21 4			
			R3	31 5	27 9	26 9								
									Average			−0 8	+2 2	+0 4

* Both feet spontaneously warm—arm baths not used.

maximum temperature observed during the pressure period and the lower represents the minimum temperature observed during the period of suction. The thermal junction recording the temperature of the air in the aluminum box was connected with the galvanometer during a complete suction and pressure cycle and the highest and lowest temperatures only were recorded. The galvanometer was of the low resistance type (Leeds and Northrup Type R) and was slightly overdamped. When relatively constant temperatures were being measured it required about 4 seconds for the galvanometer to come to rest. Positive pressure was applied for 5 seconds, a period slightly longer than that required for the galvanometer to reach equilibrium. The fluctuations in air temperature proved to be relatively uniform as shown in Figures 2B, 4-4, etc.

The air in the aluminum box was at the higher temperature for less than ¼ of the time while the lower temperature prevailed for over ¾ of the time. The average temperature of the air in the aluminum box would therefore be represented by a line slightly lower than midway between the maximum and minimum temperatures shown. Nevertheless, the air in the control box was kept warmer than the highest temperature recorded in the aluminum box since the effects of suction and pressure were being measured in terms of the cooling of the left, as compared to the right, extremity.

Under these conditions, suction and pressure modified the skin temperature of the left extremity as shown in Figure 2B. The right digits cooled uniformly and evenly during the entire period. The left digits, however, cooled more slowly during the application of suction and pressure. In the observation illustrated in Figure 2B during the early part of the suction and pressure period the left first digit was approximately 2° cooler than the right first digit. At the end of the suction and pressure period, 57 minutes later, the left first digit was approximately 3° warmer than the right. When suction and pressure were discontinued the left extremity began to cool much more rapidly and finally reached the same temperature as the right.

mum and the first five minimum readings   This figure is probably higher
than true mean air temperature for reasons mentioned above   The air in
the control box was kept approximately 2° C higher than this computed
average and slightly higher than the maximum temperature in the alu-
minium box
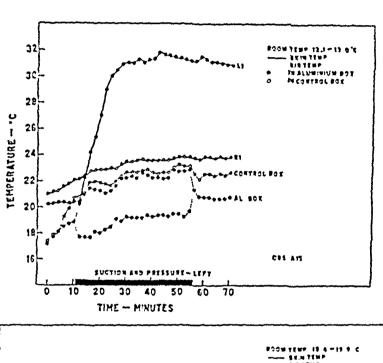
The relation between the surface temperatures of the right and left
digits is summarized in the last three columns of Table 3   The tempera-
ture of the right first digit has been subtracted from that of the left first
digit (a) at the beginning of pressure changes (b) at the end of pressure
changes, and (c) at the end of the period without pressure changes   The
third toes have been similarly compared as regards temperature
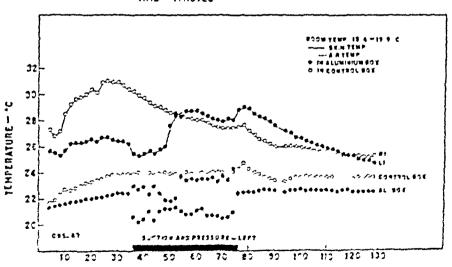
As in Table 2 a minus sign indicates that the left digit was cooler by
the amount given while a plus sign indicates that the left digit was warmer
At the beginning of suction and pressure the left digits were, with one
exception, from 0 to 1 7° C cooler than the right digits   At the end of the
suction and pressure periods the left digits were with one exception, from
0 2 to 4 5° C warmer than the right   In the two exceptions noted the
left digit cooled much less rapidly than the right, though the difference
was not enough to change the sign   During the subsequent period when
cooling continued without the application of suction and pressure to the
left extremity, the difference between the temperatures of the two extremi-
ties diminished conspicuously   In some instances the left extremity be-
came once more the cooler, in every instance the difference in temperature
became distinctly less

The results are summarized in the average figures recorded in the lower
right hand corner of Table 3   At the beginning of suction and pressure
the left digits averaged 0 8° C cooler than the right   At the end of the
suction and pressure period the left digits averaged 2 2° C warmer than
the right   After a period without suction and pressure this difference
diminished conspicuously, finally amounting to only 0 4° C   This forms
a marked contrast to the control observations recorded in Table 2 in which
without suction and pressure the left digits, instead of becoming warmer,
cooled slightly more rapidly than the right or control digits   The differ-
ence in the rate of cooling must be ascribed to the relatively greater flow
of arterial blood into the skin of the extremity exposed to suction and
pressure

*2 The influence of suction and pressure on blood flow in the cool ex-
tremities of normal subjects*

The effects on skin temperature of applying suction and pressure to
the cool extremity were observed in order to ascertain whether blood flow
could be increased in the presence of more or less marked vasoconstrictor
tone   Room temperature was low, varying from 8 9 to 19 9° C   The
subjects in these observations were usually cool or cold to the point of
shivering   As was to be expected under these conditions skin temperatures

10 observations With the beginning of suction and pressure the skin temperature of the left digits began to rise more or less rapidly, while the right digits either cooled or showed no rise in temperature other than might have been expected from the gradual warming of the control box

As shown in the upper part of Table 4 suction and pressure increased blood flow in the cold extremity in 6 of 10 observations on two normal subjects The left digits warmed by as much as 11 3° C while the right digits either cooled or warmed by not more than 2 2° C   Expressed for convenience in the form of average figures the results may be summarized by stating that in these 6 observations the left digits, exposed to suction and pressure became warmer by 6 3° C while the right digits, though exposed to a higher air temperature, became warmer by only 0 3° C
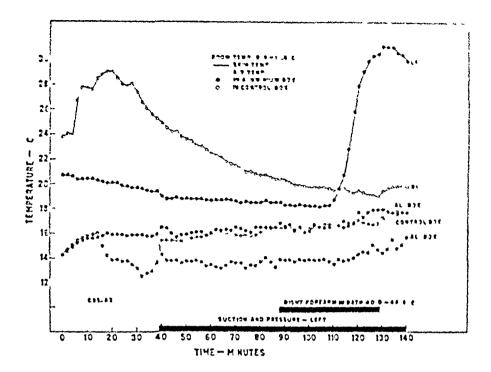
The lower section of Table 4 and the first portion of Figures 4A and 4B summarize the observations in which suction and pressure alone did not influence blood flow conspicuously The data in Table 4 apply only to the period when suction and pressure were used without immersion of the forearms in warm water, the latter being considered in the next section These four observations may be summarized by stating that the left digits exposed to suction and pressure (before diminishing vasoconstrictor tone by immersing one forearm in warm water) showed, on the average, no change in temperature while the right digits became cooler by 1° C though the latter were exposed to a warmer air temperature This difference is doubtless too small to be of any real significance, though in qualitative agreement with the other observations in which larger changes were observed High vasoconstrictor tone can apparently diminish the effects which suction and pressure exert on blood flow
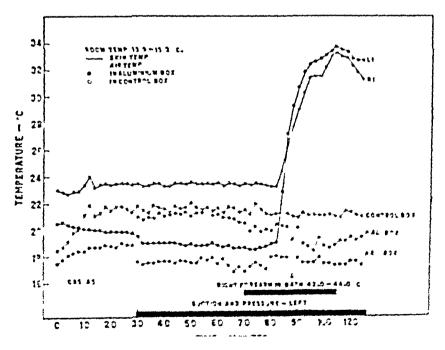
It was mentioned previously that theoretically the effects of suction and pressure would be expected to depend upon the presence of an adequate reservoir to accommodate the blood drawn through the arterioles during the suction period It has been shown by Lewis (5) that during vasoconstriction the minute vessels of the skin can resist internal positive (or external negative) pressures of 50 to 100 mm Hg The negative pressure applied to the surface of the skin was — 120 mm Hg It is very doubtful whether this pressure is transmitted without loss through the surface of the skin It is quite likely that during vasoconstriction the effective negative pressure which reaches the walls of the blood vessels could be easily withstood by vessels capable of resisting internal pressures of 50 to 100 mm Hg Under these circumstances, therefore in the absence of an adequate reservoir for the accommodation of blood flowing through the arterioles it would be expected that with high vasoconstrictor tone the effectiveness of suction and pressure would be diminished if not completely absent in some instances The effect of immersing one forearm in warm water (Figs 4A and 4B) is in favor of this explanation vasoconstrictor tone was reduced slightly a reservoir ι

TABLE 4—(Continued)

Observation number	Subject	Room temperature	Digit	Skin temperature		Duration of period of suction and pressure (left only)	Average temperature of air in boxes		Changes in skin temperature during suction and pressure	State of subject
				At beginning of suction and pressure	At end of period of suction and pressure		Beginning of pressure changes	End of pressure changes		
		C		C	C	minutes	C	C	C	
A32†	G	10 6–160	L1 L3	219 224	284 266	48	224	244	+6 5 +4 2	Chilly
			R1 R3	283 226	255 236	None	241	267	−2 8 +1 0	
A1	G	142–158	L1 L3	203 199	212 206	56	198	202	+0 9 +0 7	Cold shivering
			R1	224	225	None	215	213	+0 1	
A2	L	89–118	L1 L3	190 188	185 180	49	152	151	−0 5 −0 8	Quite chilly
			R1	252	204	None	153	163	−4 8	
A3	G	136–163	L1 L3	195 192	201 196	54	172	191	+0 6 +0 4	Cool
			R1	206	203	None	209	203	−0 3	
A5	G	139–152	L1 L3	195 197	186 189	40	193	191	−0 9 −0 8	Cold chilly
			R1 R3	235 224	233 227	None	216	215	−0 2 +0 3	

* Negative pressure, 160 to 180 mm Hg for periods of 25 seconds during the last 13 minutes
† Negative pressure, 170 to 180 mm Hg for periods of 18 seconds, with positive pressure, 70 to 80 mm Hg for periods of five seconds

suction and pressure favored blood flow through the left extremity, as
shown by the greater rise in skin temperature   The results suggest that
the maximal effects of alternate suction and pressure can be obtained only
if vasoconstrictor tone is not too great

   *3 The effects of suction and pressure on the vasodilator response to
immersing one forearm in warm water*

   Gibbon and Landis (3) showed that immersing one or two forearms
in warm water produced vasodilatation, more or less complete in grade, in
the lower extremities   In agreement with Lewis and Pickering (4) it was
observed that in general the vasodilator response began first in the warmer
extremity and that the final temperature reached at the end of 35 minutes
was slightly higher in the extremity originally the warmer
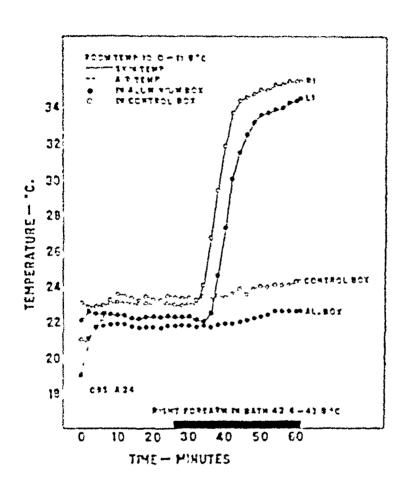
   The effects of suction and pressure on the vasodilator response were
studied, in order to determine whether blood flow was modified by external
pressure changes   Control observations were made in which the vaso-
dilator response was induced in the lower extremities without the use of
suction and pressure (Fig 5)   The left extremity was placed in the
aluminium box, the right in the control box, the air temperature in the
latter was kept uniformly higher than that in the aluminium box   In all
observations the right or control extremity was slightly warmer at the
time the forearm was immersed in the warm bath   The forearm re
mained in the bath for 35 minutes

   In Table 5A six such control observations are summarized   The av-
erage temperature of the air in the boxes was computed as in the previous
tables   In the third column from the right the temperature of a digit
was regarded as having begun to rise when it was 1° C above the tem
perature existing when the forearms were immersed   In agreement with
previous observations the right, or warmer, digits, with one exception,
showed the vasodilator response sooner than the corresponding left, or
cooler, digits   In the last two columns of Table 5A the temperatures of
the corresponding digits are compared as in previous tables   At the time
the forearm was immersed in warm water, the left digits were the cooler
by 0 4 to 1.7° C with an average difference of 1 1° C   At the end of the
35 minute immersion period the same relationship (with one exception)
existed, the left digits remaining the cooler throughout with an average
difference (including the one exception) of 1.8° C   This agrees with
previous findings and serves as a control for those observations in which
suction and pressure were used in combination with warming of one fore
arm for 35 minutes

   Figures 4A and B show that suction and pressure favored the develop-
ment of the vasodilator response in the lower extremity exposed to pressure
variations   In one observation (Fig 4A) the warmer control extremity
showed no vasodilator response whatever when the right forearm was im-
mersed in warm water while the left extremity exposed to suction and

TABLE 5—(Continued)

B With application of suction and pressure to left lower extremity during immersion of forearm in warm bath

Observation number	Subject	Room temperature C	Digit	Skin temperature — At beginning of immersion period C	Skin temperature — At end of immersion period C	Average temperature of air in boxes — Beginning of immersion period C	Average temperature of air in boxes — End of immersion period C	Time between immersion of forearm and beginning of rise in temperature (minutes)	Difference in skin temperature [L1 R1 / L3 R3] — Beginning of immersion period C	Difference in skin temperature [L1 R1 / L3 R3] — End of immersion period C
A2	L	8 9–11 8	L1	18 5	31 1	15 2	16 2	24	−1 9	+11 7
			L3	18 0	30 7	15 4	16 9	26		
A3	G	13 7–15 0	R1	20 4	19 4	15 4	16 9	No rise	−0 8	+3 3
A3	G	13 7–15 0	L1	19 7	33 9	19 0	19 5	12		
			L3	19 2	32 3			12		
			R1	20 5	30 6	20 8	21 4	16		
A5	G	13 9–15 2	L1	18 6	33 7	18 7	18 2	14	−4 8	+0 4
			L3	18 9	32 7			14	−4 0	+1 9
			R1	23 4	33 3	21 5	21 1	14		
			R3	22 9	30 8			22		
A9	G	13 1–14 7	L1	19 4	33 3	19 0	20 1	14	−1 5	+4 7
			L3	18 7	31 1			14	−1 7	+6 8
			R1	20 9	28 6	20 3	22 6	24		
			R3	20 4	24 3			30		
A20*	L	14 0–15 8	L1	20 4	33 0	22 6	23 7	7	−2 4	+1 8
			L3	20 9	31 5			15	−1 9	+1 6
			R1	22 8	31 2	24 4	25 8	9		
			R3	22 8	29 9			9		
A23*	L	12 0–13 8	L1	19 4	33 6	21 2	22 5	8	−2 4	+9 3
			L3	19 9	32 6			20	−1 7	+9 0
			R1	21 8	24 3	23 4	24 7	18		
			R3	21 6	23 6			20		
								Average	−2 3	+5 1

* Extremities colder than air in box when observation was started

The difference between the control and experimental observations can be summarized in the form of averages   Without suction and pressure the left extremity, originally cooler, began to show a vasodilator response 20 minutes after the forearm was immersed while the right, being warmer, began to show the vasodilator response in 15 minutes   The left extremity, originally 1 1° C cooler than the right, remained cooler by 1 8° C even at the height of vasodilatation   On the contrary, when suction and pressure were applied to the left lower extremity the vasodilator response appeared first in the cooler (left) extremity 15 minutes after the forearm was immersed, and in the warmer (right) extremity 20 minutes after the forearm was immersed in warm water   Moreover, while the left lower extremity was cooler than the right by 2 3° C at the beginning of the immersion period it became warmer than the right by 5 1° C at the end of the immersion period when suction and pressure were used   Suction and pressure therefore reversed the findings observed in control experiments— further evidence that negative pressure favors increased blood flow   The control observations rule out the possibility that the differences were due to chance variations in the reactions of the two extremities

C The effects of alternate suction and pressure on skin temperature in patients with peripheral vascular disease

Five patients with varying grades of peripheral vascular disease were studied   In each patient alternate suction and pressure produced a conspicuous rise of surface temperature in the affected extremity, even in the presence of advanced organic vascular disease   In these observations the room temperature was kept between 14 0 and 19 5° C —cool enough to keep air temperature within the boxes moderately low but not so cold as to be uncomfortable   The patients were in the semi recumbent position on a couch with a back rest   One lower extremity was placed in the aluminium box while the other was placed in the control box   As usual, the air in the latter box was kept slightly warmer than the air in the aluminium box   The patients experienced no discomfort during the procedure, even falling asleep in one instance while suction and pressure were applied   Skin and air temperatures were measured in the manner described for normal subjects

Patient 1, a white male, aged 61, was known to have suffered from diabetes mellitus since the age of 56   His blood sugar was well controlled by diet alone   For approximately two years the patient had noticed numbness extreme coldness and moderate cyanosis of both feet without intermittent claudication or ulceration   Examination revealed generalized arteriosclerosis with a blood pressure of 155 mm Hg systolic and 85 mm Hg diastolic. On numerous occasions both lower extremities were found to be cold and slightly to moderately cyanotic in color   The skin of the right lower extremity from the middle of the leg downward was thickened and scaly and all the toe nails were abnormally ridged and thickened   Neither
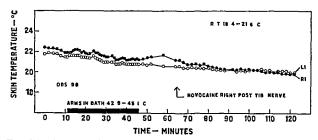
FIG 6A   SHOWING COMPLETE ABSENCE OF VASODILATOR RESPONSE IN PA-
TIENT 1 DURING (1) IMMERSION OF ARMS IN WARM WATER AND (2) DURING
ANESTHETIZATION OF THE RIGHT POSTERIOR TIBIAL NERVE
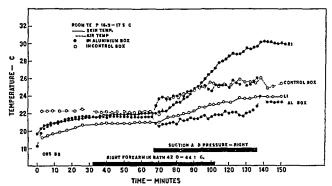
FIG 6B   SHOWING RISE IN SKIN TEMPERATURE OF THE RIGHT LOWER
EXTREMITY DURING APPLICATION OF SUCTION AND PRESSURE
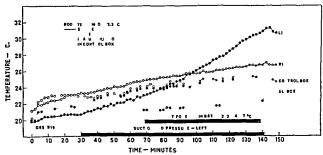
FIG 6C   SHOWING RISE IN SKIN TEMPERATURE OF LEFT LOWER EXTREMITY
DURING APPLICATION OF SUCTION AND PRESSURE

TABLE 6—(Continued)

Patient number	Observation	Room temperature	Digit	Skin temperature		Duration of suction and pressure	Average temperature of air in boxes		Amount of warming	Rise in skin temperature
				At beginning of period of suction and pressure	At end of period of suction and pressure		Beginning of pressure changes	End of pressure changes		
		C		C	C	minutes	C	C		C
3	B20	14 9-16 0	R1 R3	20 0 / 21 7	32 3 / 27 4	56	22 9	24 9	None	12 3 / 5 7
			L1 L3	21 2 / 21 7	24 0 / 23 8	None	25 3	27 0		2 8 / 2 1
4	B15	15 7-18 0	R1 R3	27 1 / 26 4	31 5 / 30 0	27	21 6	22 3	None	4 4 / 3 6
			L1 L3	31 1 / 29 5	30 4 / 29 1	None	24 2	24 6		−0 7 / −0 4
5	B2	16 0-17 0	R1 R3	24 0 / 24 4	30 3 / 32 3	118	23 5	25 8	One forearm	6 3 / 7 9
			L1 L3	24 4 / 24 9	28 4 / 25 9	None	24 6	27 0		4 0 / 1 0
5	B7	18 2-19 0	R1 R3	26 6 / 26 8	29 4 / 31 4	53	25 1	25 6	None	2 8 / 4 6
			L1 L3	31 2 / 29 0	29 6 / 28 4	None	26 6	27 5		−1 6 / −0 6
5	B17	14 4-16 0	R1 R3	22 2 / 22 7	24 9 / 24 0	51	20 8	22 1	None	2 7 / 1 3
			L1 L3	22 6 / 23 0	23 4 / 23 2	None	22 4	24 3		0 8 / 0 2
5	B21	14 0-15 1	R1 R3	19 5 / 19 2	32 1 / 31 7	81	20 0	21 9	One forearm	12 6 / 12 5
			L1 L3	19 9 / 19 6	23 0 / 21 9	None	22 4	24 3		3 1 / 2 3

posterior tibial nerve and the right first, third and fifth digits, originally 22 1, 21 6, and 20 5° C warmed to 32 5, 32 9 and 31 7° C respectively in a room at 19 to 20° C   This response indicated that the symptoms were due primarily to arterial spasm

At a room temperature of 14 9 to 16 0° C the right lower extremity was placed in the aluminium box, the left lower extremity in the control box   Suction and pressure were applied to the right lower extremity for 56 minutes   At the beginning of this period the right first and third toes were slightly cyanotic and slightly cooler than the left toes   During the period of pressure changes the right digits became definitely pink and warmer by 12 3 and 5 7° C while the left digits became warmer by 2 8 and 2 1° C, the latter being exposed in the control box to an average air temperature over 2° C higher than that in the aluminium box   The right digits exposed to suction and pressure, became conspicuously warmer than the surrounding air while the left digits originally cooler than the surrounding air remained so throughout the observation

Patient 4 (B1), a white male aged 46, was a diabetic whose right second toe had previously been amputated on account of gangrene   The tip of the right third and fourth toes had been the seat of gangrenous ulcers which healed with rest and local heat   On two occasions immersing the forearms in warm water failed to produce vasodilator response, indicating marked organic obstruction, verified in the left foot by the absence of vaso dilatation after anesthetizing the left posterior tibial nerve   This patient's history has already been summarized in another paper (3)

The right lower extremity was placed in the aluminium box, the left in the control box   The temperature of the left foot was higher than that of the right   both extremities being relatively warm because the patient had been brought straight from the ward where the feet were kept constantly under a warm cradle   Suction and pressure were applied to the right lower extremity for 27 minutes and during this period the right first and third digits became 4 4 and 3 6° C warmer while the corresponding digits of the other foot became 0 7 and 0 4° C cooler respectively, though the latter were exposed to environmental temperature over 2° C higher   The right digits originally cooler than the left, became, during suction and pressure, warmer than the left

Patient 5, a white Hebrew male, aged 58, suffered from thromboangitis obliterans with coldness and cyanosis of the right foot as well as typical intermittent claudication in the right leg   The left foot became cold easily but cyanosis and pain were absent

The posterior tibial pulse was absent bilaterally, the dorsalis pedis pulse was absent on the right but present on the left   Immersing both forearms in warm water on several occasions indicated uniformly that the arteries of the right extremity were organically diseased to a conspicuous degree while those of the left extremity were less conspicuously affected   The vasomotor index of the right foot was 1 1, that of the left 1 3

observation charted in Figure 7A the left extremity was exposed to room air having a temperature of 18 8 to 20 1° C, the right lower extremity being placed in the aluminium box with an air temperature of approximately 24° C   Immersing one forearm in warm water was followed by a relatively rapid warming of the left first toe to 32 2° C while the right first toe in spite of the higher temperature of the surrounding air warmed more slowly to 28 1° C during the same period

The effects of suction and pressure on skin temperature in this patient are shown in Figure 7B   The left and more nearly normal extremity was placed in the warmer control box   The right, more diseased extremity was placed in the cooler aluminium box   Suction and pressure alone failed to change skin temperature appreciably at the relatively low room temperature of 14 0 to 15 1° C   The right forearm was immersed in warm water and after approximately twenty minutes the temperature of the right first toe rose above that of the left and finally reached 32 1° C   The left first toe though originally the warmer, and in a warmer environment throughout the observation, remained cooler by 9 5° C   Four observations on this patient are summarized in Table 6   In each instance the right digits, originally cooler than the left, became warmer than the left during suction and pressure and the ordinary temperature relationship shown in Figure 7A in response to heat alone was reversed

If all the figures in Table 6 are summarized in the form of averages it is found that the digits exposed to suction and pressure became warmer by 7 1° C while the control digits became warmer by 1 9° C although the latter were in a warmer environment   From these averages and from the individual observations it appears that applying suction and pressure increases the flow of blood to the digits even in the presence of organic arterial disease   The findings in Patients 1, 2 and 4 indicate that suction and pressure may increase blood flow though removal of vasoconstrictor tone fails to do so

D Skin color during suction and pressure

In this paper emphasis has been laid upon changes in skin temperature since they seemed theoretically to provide the most objective and convincing means of identifying changes in arterial blood flow   Skin color was also observed but drawing definite conclusions on this basis alone did not seem justifiable for several reasons   When the extremities are cold as emphasized by Lewis (6) in his studies of Raynaud s disease, cyanosis develops relatively slowly   As regards blood flow redness or cyanosis of the skin with surface temperature of 30° C has an entirely different significance from the same grade of redness or cyanosis at a surface temperature of 20° C   Moreover, skin color depends to a certain extent, as shown by Goldschmidt and Light (7), upon the amount of blood in the skin   Therefore, diminution of cyanosis was regarded as corroborative but not

and pressure to the left extremity was accompanied by a lesser cyanosis in the left first and third digits as compared to the right first and third digits

In Patient 2 immersing both forearms in warm water produced neither a lessening of cyanosis nor an increase in skin temperature in the lower extremities   Negative and positive pressures caused the right first digit and the dorsum of the right foot to become definitely pink though both were cyanotic before the pump was started   An area of skin over the first metatarso phalangeal joint though deeply cyanotic before the application of suction and pressure was normal in color at the end of the observation, while the corresponding area in the left foot was still cyanotic

In Patient 3 the right first digit, moderately cyanotic before the application of suction and pressure, was definitely pinker at the end of the observation   In Patient 4 no color observations could be made due to unsatisfactory light

In Patient 5 the skin color of the right digits, originally moderately cyanotic, changed but little when vasoconstrictor tone was reduced by immersing both forearms in warm water   In four observations (B2, B7, B17 and B21) the color of the right digits became brighter, changing from a moderate or marked cyanotic tint before suction and pressure to a normal tint or at most a slightly cyanotic tint at the end of the suction and pressure period

The observations on skin color corroborated the more objective and definite skin temperature studies both indicating an increased blood flow during the application of alternate suction and pressure

DISCUSSION

The desirability of increasing local blood supply in certain medical and surgical conditions involving the extremities has long been recognized As an early attempt in this direction the application of a bandage proximal to the lesion was, according to Bier (8), first described by Paré (9) This method was later used chiefly for treating ununited fractures by Nicoladoni (10), Thomas (11), and Helferich (12) but has been more recently revived and extended by Bier (8)   While such passive congestion produces venous engorgement and increases the amount of blood trapped in the vessels distal to the bandage it does not increase, but really reduces total blood flow if congestion is extreme, as shown by the calorimetric measurements of Stewart (13)

Negative pressure was apparently first extensively used in 1829 by Junod (14) who constructed a series of pneumatic chambers ' designed to accommodate one or more extremities or even most of the body   Under the name of ' hemospasie " negative pressure was used enthusiastically to treat almost every form of local or systemic disease   Junod used negative pressures amounting to between ⅓ and ½ atmosphere over more ~~ less

tightness of the skin when vasoconstrictor tone was reduced by warming the forearms   When vasodilatation was not extreme 25 seconds of negative pressure produced little sensation of distention   In all of the observations on normal subjects and patients discomfort has been negligible and no hemorrhages into the skin have been observed   A negative pressure of 120 mm Hg would undoubtedly produce cutaneous hemorrhages if it were permitted to act for a long time   However, during periods of 25 seconds the veins and capillaries are not maximally distended and consequently the vessel walls are not stretched to a point where hemorrhages are apt to occur   Moreover, in patients with organic arterial obstruction distention would occur at a later period than in normal subjects whose arteries are still of normal calibre

The cuff congests the leg slightly and in itself tends to keep the veins distended   Hence brief periods of pressure were used to empty the peripheral vessels and to provide an adequate reservoir for the accommodation of the blood drawn in by the succeeding period of suction, an essential point theoretically in favoring an increase in the rate at which arterial blood enters the capillaries and veins   Simple intermittent suction with rest periods would probably be less efficient since (a) the congestion produced by the cuff would not be overcome and (b) emptying would be slow and incomplete   The importance of diminishing vasoconstrictor tone has already been considered

Inspection of the tables will show that occasionally skin temperatures were lower than the temperature of the air in the boxes   At the outset the air in the boxes had the same temperature as room air   As soon as the boxes were closed the air surrounding the extremities began to warm presumably due to radiation from the warmer proximal portions of the extremity enclosed within the boxes   Nevertheless, the temperature of the digits exposed to suction and pressure rose well above environmental temperature

The possibility that the rise in skin temperature was due purely to physical forces incident to changing air pressure can be ruled out by inspection of Figures 4A, 4B, 6C, and 7B   If the elevation of skin temperature had been due to condensation of moisture or to heat produced during compression of the air within the box the skin temperature would have begun to rise in every instance as soon as the pressure variations were used   In these four observations as well as many others skin temperature did not change, in spite of suction and pressure, until vasoconstrictor tone was diminished   Moreover, such physical effects could not explain the elevation of skin temperature above the environmental temperature   A bottle covered with moist gauze the latter to simulate moist skin, cooled slightly in the aluminium box during the prolonged application of positive and negative pressure   These observations are believed to rule out artefacts due to purely physical factors

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PROCEEDINGS OF THE TWENTY–FIFTH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION HELD IN WASHINGTON, D C, MAY 8, 1933

Treatment of Pellagra with Liver Extracts By DAVID T SMITH and (by invitation) JULIAN M RUFFIN, Durham N C

During the past two years a series of pellagra patients have been studied at the Duke Hospital A basic diet was planned which contained all the normal necessary vitamins and minerals except vitamin B2 or G The patients maintained on this diet failed to make satisfactory improvement until vitamin G in the form of liver extract was added to the diet It has been demonstrated that the symmetrical skin lesions in pellagra are due to symmetrical exposure to sunlight and have no relation to nerve lesions in the spinal cord Unilateral skin lesions could be produced at will by proper exposure to sunlight Experimental black tongue in dogs has been produced with the same diet used as a basic diet for the clinical studies This experimental black tongue can be cured by liver extracts

The Urinary Excretion of Iodine By GEORGE M CURTIS and (by invitation) FRANCIS J PHILLIPS, Columbus, Ohio

The urinary excretion of iodine by hospital patients without thyroid disease in this region is from 25 to 75 microgrammes daily No iodized salt is used in the hospital diet It is within normal range in patients with non toxic nodular goiter and also in those with non toxic diffuse colloid goiter In patients with diffuse hyperplastic goiter with exophthalmos and with severe hyperthyroidism, it is greatly increased during the early exacerbation of severe, untreated toxicity The urinary excretion of iodine increases greatly immediately following thyroidectomy This increase is temporary When iodine is quantitatively administered to patients with diffuse hyperplastic goiter with hyperthyroidism, the urinary excretion is at first low There ensues a rising iodine excretion curve as clinical improvement follows The urinary excretion of iodine is increased during early menstruation This is correlated with the associated rise in the blood iodine

Congenital Heart Block Review with Report of Detailed Histo Pathologic Study of the Second Case of Complete Congenital Heart Block Studied by Serial Sections Through the Conduction System By WALLACE M YATER and (by invitation) JAMES A LYON and PAUL E McNABB Washington D C

Cases of congenital heart block are not common About forty cases have been reported in the literature which fulfill certain arbitrary criteria for the making of this diagnosis The cause has usually been   patent interventricular septum with anatomical break in the au ̲   le
However, necropsy findings of only four cases have been ι๛   ᵥ
only two were studied by serial sections through the conduction
was a case of 2 to 1 heart block in which there was almost   of
the interventricular septum the other was a case studied by Yater

46   963

heart block in which there were very unusual anomalies but in which a septal defect did not play a rôle   The case now reported is one of an infant who had a defect in the interventricular septum in its middle upper portion   There was found a complete interruption of the bundle of His by the defect   In fact, practically the whole bundle was missing   The electrocardiogram, however, showed the QRS complex to be of the supraventricular type   The diagnosis of complete heart block due to patent interventricular septum was made during life

Significance of Inorganic Sulphate Coefficient or Clearance in Renal Disease
    By JOHN W MACY (by invitation) and NORMAN M KEITH, Rochester, Minn
    Inorganic sulphate concentration was determined in blood serum and urine during a given period of urine excretion   The coefficient or clearance was determined by the following formula

$$\frac{\text{Per cent sulphate concentration in urine}}{\text{Per cent sulphate concentration in blood serum}} \times \text{minute volume of urine}$$

In twelve normal individuals the sulphate coefficient or clearance was very constant from hour to hour, the variation being less than in the corresponding urea and creatinine coefficients   This fact suggests that the inorganic sulphate coefficient might be useful as a standard with which the coefficient of other substances might be satisfactorily compared
    In renal disease the sulphate coefficient is in general lowered in proportion to the degree of insufficiency   In some cases of this series, the sulphate coefficient appears to be decreased when other functional tests, including urea clearance, are within normal range   This suggests that the sulphate coefficient or clearance may have an important place in those cases in which early renal insufficiency is suspected

The Effect of Gelatin Diets in Nephritis   By G P GRABFIELD, Boston, Mass
    Previous studies of nephritis have indicated that there is some alteration in patients with Bright's disease in their handling of sulphur, more especially in relation to the nitrogen   Therefore, in the present study it was decided to try the effect of sulphur-poor diets on patients with Bright's disease   The problem of providing adequate amounts of nitrogen without sulphur was solved by using gelatin as the source of practically all the dietary nitrogen   In practice, standard nephritic diets were used, patients were studied for three-day periods and during the test period, practically all the protein of the diet was given to the patient in the form of gelatin in the same amount as the previous protein of the diet   These diets were difficult to take and the only cases reported are those which succeeded in eating all the diet
    Unfortunately, few control experiments of this type are recorded in the literature but those that are, indicate that gelatin is an incomplete protein and is only partly utilized   The degree of utilization varies and the estimates are that from 11 to 40 per cent must be made up by the breakdown of body protein   The interesting thing about the figures as shown, is that if we take the sulphur as an index of the amount of nitrogen metabolized, it remains essentially the same during the gelatin periods as previously   As these diets were adequate except for the protein and sulphur, it seems to us that this may be taken as an indication that there is an alteration in the utilization of sulphur in these patients with Bright's disease   In other words, these figures indicate that the

amount of the sulphur rich moiety of the protein molecule that is metabolized in such patients is only partly dependent upon the diet

The Effects of Posture (Standing) on the Serum Protein Concentration and Colloid Osmotic Pressure of Blood from the Foot in Relation to the Formation of Edema By John B Youmans and (by invitation) H S Wells Dorothy Donley, and D G Miller, Nashville, Tenn

The influence of posture on the serum proteins and osmotic pressure of blood in the foot was studied with reference to formation of edema, particularly in persons with slightly lowered serum proteins Others have shown a general concentration of blood in the erect posture, apparently due to loss of fluid from the vessels in the dependent tissues but protein concentration and osmotic pressure of blood from the foot have not been determined Normal subjects after standing approximately an hour showed a 17 to 40 per cent concentration of proteins and a 24 to 64 per cent increase in osmotic pressure in blood from the foot In all cases the rise in osmotic pressure was relatively greater than the concentration of protein Concentration was much greater in the foot than in the arm The escape of fluid was indirectly indicated by an increase in leg volume but pitting edema was absent in most normals Patients with edema showed an increase in proteins of 14 to 33 per cent and in osmotic pressure of 23 to 46 per cent but the average increases were less than in normals while the increase in leg volume was greater and the edema was demonstrably increased

A Study of the Nasal Secretions in Disease of the Upper Respiratory Tract By Perrin H Long and (by invitation) Eleanor A Bliss and Harriet M Carpenter, Baltimore, Md

Studies of the nasal secretions during " colds " have shown that in the early hours of a " cold ' many epithelial and mononuclear cells are found By the third day the cellular content of the nasal secretions is chiefly made up of polymorphonuclear leukocytes In many instances stained slide preparations of the nasal secretions late in ' colds " failed to show appreciable numbers of bacteria It is suggested that the cellular reaction of the nasal secretions in colds " is a response to the infecting filterable agent rather than to the bacterial flora of the nasal passages

Contraction of the Spleen in a Case of Anemia with Splenomegaly By D K Miller (introduced by C P Rhoads) New York City, N Y

Contraction of the spleen was studied during the intravenous injection of liver extract in a patient with anemia and splenomegaly This afforded an opportunity to ascertain certain facts regarding the function of the human spleen as a reservoir of red and white corpuscles and blood platelets

The spleen was visualized by x ray Its area was measured before and after the injection of liver extract The contraction occurred during the injection, which was given over a period of twenty minutes The spleen regained its original size after one half to six hours

In a typical study the surface area of the spleen as measured from the x rays with a Keuffel and Esser planimeter was 90 3 sq cm before the injection Immediately after the injection the surface area was 32 3 sq cm Twenty four hours later the area was 89 1 sq cm Accompanying this contraction there was an increase in the red cell count of 15 1 per cent. The hemoglobin increased 21 5 per cent, and the white cell count 54 0 per cent An

increase in the number of blood platelets of 120 per cent occurred   The fragility of the red cells to sodium chloride solution was not altered

Blood volume determinations by the vital red method of Rowntree showed no appreciable alteration   There was a decrease in the plasma percentage of 7 7 per cent

From data of this nature it has been possible to estimate the relative cellular composition of the blood given off from the splenic reservoir into the general circulation as a result of induced and observed contractions of the spleen

The Nature of the Muscular Weakness in Graves' Disease   By E SHORR, H B RICHARDSON and H G WOLFF, New York City, N Y

Muscular weakness is a common symptom in Graves' disease   In such patients a very constant abnormality is the presence of excessive amounts of creatin in the urine   Creatin in muscle has been shown to be combined with phosphoric acid as a labile compound, phosphocreatin (Fiske and Subbarow), now considered to be of primary significance for muscular contraction   From experiments on certain pathological states, it appears probable that glycin is an important precursor of creatin   This amino-acid is readily synthesized in the body, chiefly in the liver

Recently, considerable information has been obtained concerning the relation between the muscular weakness and the disturbance of creatin metabolism in progressive muscular dystrophy   Since the muscular weakness in Graves' disease is often very great, and a creatinuria is common to both conditions, the creatin metabolism of patients with Graves' disease was investigated   Several analogies between the two syndromes were revealed   Thus, in both (1) the ability to retain ingested creatin (presumably as phosphocreatin) was markedly diminished, (2) the ingestion of glycin resulted in large increases in creatin elimination, frequently greater in Graves' disease than in advanced cases of progressive muscular dystrophy   In some instances the rise was transitory, falling to lower levels or disappearing with continued glycin feeding, (3) the ability to form glycin, as measured by hippuric acid excretion after the administration of sodium benzoate, was unimpaired   Furthermore, following ingestion of sodium benzoate, there was frequently observed a sharp increase in the creatin output, the significance of which is yet unknown

An additional point of resemblance was furnished by the observation that long-standing cases of Graves' disease showed anatomical changes in the skeletal muscle similar in many respects to those found in progressive muscular dystrophy (Askanazy)

Following the administration of iodine to patients with Graves' disease, as was previously observed (Palmer et al), there was a progressive fall in creatinuria to the normal level, usually accompanied by a fall in the basal metabolism   During the latter phase, we gave creatin and glycin again, but now no increase in creatin output resulted   This is analogous to what occurs in progressive muscular dystrophy after prolonged feeding of glycin   Additional ingestion of glycin and creatin then produces no increase in creatin output (Thomas, Milhorat and Techner)

The factors causing these disturbances of creatin metabolism in Graves' disease are still obscure   It is postulated that the processes maintaining the integrity of the phosphocreatin mechanism are taxed too severely by the persistently high metabolism   One of these, the endogenous formation of glycin, was found to be normal   However, though adequate in proportion to the body weight and the normal basal metabolism, its production may be low in proportion

to the needs of an elevated total metabolism   The increased demands resulting from the latter may lead to a progressive depletion of the elements involved in the phosphocreatin system   Whether iodine acts directly on this muscle mechanism in addition to reducing the basal metabolism, cannot be stated

Conclusion   The muscular weakness in Graves' disease is the result of a reparable impairment of the phosphocreatin mechanism, and is of the nature of an acute muscular dystrophy similar in many respects to the disturbance in progressive muscular dystrophy

The Effect of Convulsant Drugs on the Cerebral Vessels   By STANLEY COBB and (by invitation) JACOB E FINESINGER Boston, Mass

This paper is a report of microscopical observations through a window in the skull of the small arteries of the pia, and of their reactions after the administration of convulsant drugs to the animal under observation   The drugs used were caffeine wormwood oil homocamfin camphor monobromate and picrotoxin   The intravenous introduction of large doses of caffeine produced an acute constriction of the pial arteries followed by a generalized convulsion   Small doses of wormwood oil gave a slight constriction of the pial artery followed by a generalized convulsion   large doses of wormwood oil produced a dilatation of the pial arteries before the onset of the generalized convulsion   Homocamfin and picrotoxin produced a slight constriction of the pial artery preceding the convulsion   Camphor monobromate gave a dilatation of the pial arteries preceding the convulsion   These experimental convulsions were not preceded by a consistent type of vascular response to the drugs used

The Nonprotein Iron of the Blood   By J F McINTOSH Peiping China

Relatively little attention has been paid to that part of the iron of the blood which is not contained in the hemoglobin molecule   Most investigators have considered it as negligible   Scarcely any determinations of it have been made in clinical cases

The nonprotein iron of whole blood has been determined directly by analysis of the trichloracetic acid filtrate   In normals the average value found was 1 02 mgm per 100 cc   A variety of anemic cases were studied, including pernicious anemia achlorhydric hypochromic anemia and secondary anemias due to hemorrhage cancer and various infections   In these, the nonprotein iron was reduced in proportion to the degree of anemia   The different diseases do not show any grouping which deserves emphasis

The greater part of the nonprotein iron is to be found in the red cells

The Case of Captain Charles Martell What It Has Taught Us About Generalized Osteitis Fibrosa Cystica   By WALTER BAUER and (by invitation) CHARLES L SHORT, Boston Mass

The diagnosis of generalized osteitis fibrosa cystica was first made in this country by DuBois in 1926   Through the kindness and generosity of Dr Du Bois we have been privileged to study this patient, Captain Charles Martell, at various times during the ensuing six years   Because of his courage interest in clinical investigation and desire to live as well as to help others live he permitted detailed clinical and metabolic studies on numerous occasions submitted to eight operations and asked that a complete necropsy be done at the time of his death

A detailed review of these studies reveals not only the clinical and metabolic course of generalized osteitis fibrosa cystica, its complications, the metabolic changes resulting from these complications, but also demonstrates the difficulty that may be encountered in finding the offending parathyroid tumor in this disease, as well as the pathological changes resulting from hyperparathyroidism of fifteen years duration

The Conversion of Glycerol to Glucose by the Animal Organism   By F H LASHMET (by invitation) and L H NEWBURGH, Ann Arbor, Mich
In its metabolism, 10 per cent of fat is converted to glycerol   On the basis that 100 per cent of glycerol is converted to glucose, it has been customary to consider 10 per cent of the fat of the diabetic diet as available glucose
The data, upon which the conclusion is based that glycerol is completely converted to glucose, are very unconvincing, even as originally presented, and especially so when recalculated
Our investigation, employing more recent methods than were available to older observers, seems to show that less than 30 per cent of glycerol is converted to glucose   If our work is substantiated, it is evident that only 2 or 3 per cent of fat is converted to glucose

Studies on the Origin of Plasma Proteins   By HOBART A REIMANN and (by invitation) GRACE MEDES and LUTHER C FISHER, Minneapolis, Minn
Previous experiments indicated that an increase of blood viscosity due to increase of globulin and fibrinogen caused enhancement of the agglutination of bacteria   Studies were then made to locate the source of these proteins which appear to be associated with the immune defense mechanism   There are four theoretical sources of fibrinogen   (a) liver, (b) erythrocytes, (c) bone marrow, (d) leukocytes   Considerable presumptive evidence favors the latter two, especially the leukocytes
Leukocytes were obtained by injecting aleuronat or gum acacia into the pleural cavities of rabbits   The cells were disintegrated by freezing with liquid air and triturating in a mortar   The resulting viscid mass was redissolved in salt solution, dialyzed and fractionated with sodium sulfate   Proteins with the salting-out characteristics of fibrinogen, globulin and albumin were present   Immunologic tests are under way to test the identity of these proteins with their counterparts in the plasma
Heavy suspensions of leukocytes in broth were placed in cellophan capsules and embedded subcutaneously in rabbits   After 4 to 7 days the capsules were removed   The leukocytes had largely disintegrated and globulin- and fibrinogen-like proteins were detected in the broth   Leukocytes were then destroyed in vivo by benzol injections or by exposure to x-rays   In one benzol experiment the globulins and fibrinogen increased markedly as the number of circulating leukocytes fell   During the period of aplasia of the marrow, the fibrinogen diminished   Other experiments failed to give striking results   In general, the fibrinogen tended to increase, and the total protein to diminish at the expense of the albumin fraction   The marked leukopenia produced by x-rays caused but little change in the blood proteins   The fibrinogen increased somewhat immediately following the diminution of leukocytes
These experiments indicate that globulin and fibrinogen may in part be derived from the decomposition of the leukocytic cells, especially of the polymorphonuclear type in the circulation or in the hematopoietic system

Allergy and Immunity in Tuberculosis By JONAS S FRIEDENWALD and (by invitation) HERBERT ROTHSCHILD and CLARENCE BERNSTEIN, Baltimore, Md

A group of guinea pigs was made immune to tuberculosis and allergic to tuberculin by inoculation with a virulent strain of tubercle bacilli When allergy had been well established one half of the animals were desensitized by daily subcutaneous injections of Koch s O T in increasing doses When allergy had been completely suppressed, the desensitized animals together with the allergic animals and a group of normal controls were inoculated in the groin, in the eye and in the skin with a virulent strain of tubercle bacilli The injections of tuberculin in desensitized doses were continued daily The results showed that the loss of allergy by desensitization was not accompanied by any loss of immunity

Heterologous Scarlet Fever By JAMES D TRASK and FRANCIS G BLAKE, New Haven Conn

Two cases of scarlet fever which failed to respond to large doses of therapeutic scarlet fever antitoxin were found to have a heterologous toxin in their blood The blood serum from these patients collected during the active stage of the disease elicited strongly positive skin reactions in certain Dick negative test subjects and no reaction in Dick positive subjects In vitro the serum was neutralized by the blood serum of the Dick positive individuals but not by that of the Dick negative individuals

A toxin was prepared from the hemolytic streptococci isolated from the two patients Both in the reactions it elicited on intracutaneous injection and by the results of neutralization tests it corresponded with the toxin in the blood samples mentioned above

Accordingly, the above results taken in conjunction with our previous studies show that heterogenicity exists among the toxins found in the blood during scarlet fever and among the antitoxins found in the blood serum of normal individuals

Electrocardiograms that Record the Potential Variations Produced by the Heart Beat at a Single Point By PAUL S BARKER FRANK N WILSON and (by invitation) FRANKLIN D JOHNSTON, Ann Arbor, Mich

A method has been devised which makes it possible to record the potential variations produced by the heart beat at a single point This method has been used to explore the electric field produced by the heart at the body surface in bundle branch block ventricular hypertrophy coronary occlusion and other cardiac conditions By this means, it is possible to compare the potential variations that occur at points near the heart with the potential variations of the extremities In general it is found that the potential variations of a given extremity are similar to those that occur on those surfaces of the heart that face toward the attachment of that extremity In the common type of bundle branch block and in left ventricular enlargement the potential variations of the left arm are similar to those that occur over those portions of the precordium that overlie the left ventricle, the potentials of the left leg are similar to those that occur over those portions of the precordium that overlie the right ventricle Characteristic curves are obtained from the precordium when the anterior surface of the heart is infarcted

The Effect of Vagus and Sympathetic Stimulation on the Coronary Flow in Revived Human Hearts By WILLIAM B KOUNTZ (introduced by David P Barr), St Louis, Mo

Human hearts have been revived in the body by perfusion   The hearts were perfused through an aortic cannula until they began to beat strongly Cannulae were then inserted into the right coronary artery and into the circumflex branch of the left coronary artery   These were then connected to a reservoir of perfusion fluid, the height of which could be regulated so as to obtain any desired pressure   In some experiments another reservoir containing Locke's solution free of calcium or with excess of calcium was arranged in the perfusion circuit   The flow of fluid from the reservoirs was measured by a volumetric recorder

When the coronary vessels were perfused with buffered Locke's solution containing at least 15 per cent blood, stimulation of the peripheral end of the vagus nerve slowed the heart rate and increased the coronary flow   Stimulation of the sympathetic produced exactly opposite effects under the same circumstances

Further analysis was made by stopping the heart through removal of calcium from the perfusing solution or through the addition of an excess of calcium   In some experiments changes in the pH of the perfusion fluid in the range from 7 0 to 7 8 were made

It was found that when hearts were stopped by removal of calcium or by reducing the pH of the perfusion fluid to about 7 0 vagus stimulation had no influence on the coronary flow   Sympathetic stimulation on the other hand increased the flow   When the hearts were stopped by an excess of calcium or by increasing the pH to about 7 8 vagus stimulation increased the coronary flow, while sympathetic stimulation had no influence upon it

In the beating heart the mechanical factor of change in heart rate makes it difficult to evaluate the effect of the sympathetic and vagus activity on the heart   It is suggested that changes in the coronary flow in the arrested hearts may be due to change in tone

Further Observations on the Use of Chest Leads in the Electrocardiographic Study of Coronary Occlusion By CHARLES C WOLFERTH and (by invitation) SAMUEL BELLET, THOMAS M McMILLAN, and FRANCIS CLARK WOOD, Philadelphia, Pa

Fifty-seven cases of acute coronary occlusion and 280 controls, both normal and pathological, were studied with chest leads and limb leads   The following facts developed

1 Twenty-three of our fifty-seven cases of acute coronary occlusion showed signs of recent cardiac infarction in an anteroposterior chest lead (Lead IV) and not in limb leads, at some time during their course

2 Three types of left ventricular infarction have been seen at necropsy the anterior (5 cases), the posterior (1 case) and the lateral (1 case)   Lead IV helps to differentiate these types electrocardiographically   In our series of 57, 33 were anterior, 18 were posterior, 5 were probably lateral, and 1 was not classified

3 Huge upright T-waves in Lead IV probably signify acute or subacute anterior infarction

4 The advantage of Lead IV over limb leads is seen mainly in the study of cases of anterior infarction   Twenty-one of thirty-three acute anterior cases, at some time during their course, showed diagnostic findings in Lead IV and not

in limb leads Moreover, Lead IV sometimes helps to differentiate T–1 inversions produced by healed anterior infarction from those due to other causes

5 In our cases the pain of anterior infarction has usually been situated lower in the chest than the pain of posterior infarction

6 Anterior infarction seems to have a less favorable prognosis for life and for adequate recovery than posterior infarction

7 A standard normal electrocardiogram in the anteroposterior chest lead is as readily obtainable as a standard in limb leads

8 S–T interval deviations caused by conditions other than acute coronary occlusion are if anything less confusing when chest leads are used with limb leads They rarely resemble the typical electrocardiographic picture of either of the two common types of coronary occlusion

Bone Lesions in Yaws By THOMAS B TURNER and (by invitation) GEORGE M SAUNDERS Baltimore Md

Approximately 10 per cent of 1100 yaws patients studied in Jamaica exhibited lesions of the bones, although among individuals infected less than five years the incidence was 14 per cent The characteristic lesion as revealed by roentgen ray consists of multiple small areas of rarefaction in the cortex with or without periosteal proliferation rarely periostitis occurs alone Often there is new bone formation about the areas of rarefaction giving rise to clinically recognizable enlargement and deformity of the bone The long bones are chiefly affected The onset of typical lesions has been observed as early as one month and as late as fifteen years after infection with yaws Persistent dull pain is the most prominent symptom and active lesions may persist for several years eventually healing spontaneously or extending to involve the overlying soft parts with subsequent development of chronic ulcers If not too far advanced there is prompt response to anti yaws treatment Photographs of the clinical and roentgenological appearance of typical bone lesions were shown

Preliminary Report on the Treatment of Chronic and Subacute Infectious Arthritis by Artificial Fever By LAWRENCE A KOHN and (by invitation) STAFFORD L WARREN Rochester, N Y

Forty three patients in the main with purely atrophic, proliferative, and some mixed types (infectious and degenerative arthritis), in various stages of disability deformity, and chronicity (six months to fourteen years) have been subjected to one or two treatments of artificial fever No cases of gonorrheal origin or purely degenerative arthritis have been included Twenty one patients were treated in 1931 of these five have not returned in 1933 (one was not improved and the rest were improved when last heard from after 1 to 14 months) Twenty two patients were treated in 1932 (of these two have not returned and one was unimproved one month later and has been lost sight of) The results have been quite consistent in thirty five patients, in all but two of whom pain has rapidly diminished and mobility of joints has increased a steady improvement in the condition of the patient a gain in weight (2 to 17 kgm), and disappearance of pallor have occurred in the following four to six months

Relapses have occurred at the end of 12, 20 and 24 months respectively in three patients in whom another treatment has been successful in giving relief Eight incapacitated patients have been able to earn a living

Antibody Response to Pneumococcic Infections   By MAXWELL FINLAND and
    ALEXANDER W WINKLER (introduced by Chester S Keefer), Boston,
    Mass

One hundred and fifty-eight patients from whom pneumococci of various
types (Cooper) other than Type I were isolated were studied with reference
to the formation of antibodies   Of these, 110 were patients with pneumonia
and the remainder had other acute or chronic respiratory infections   Tests for
serum agglutinins were carried out before and after crisis by the use of anti-
gens prepared from a variety of type-specific pneumococci   Each serum was
tested with one or more antigens of the type homologous with that recovered
from the patient and, in addition, against a number of antigens of heterologous
types   In the case of Types II, III, V and VIII protection tests were also
carried out.

The results showed that agglutinin formation can be demonstrated in the
course of pneumonia and occasionally of other infections associated with the
finding of some of the new types of pneumococci   The specificity of the anti-
body response is similar to that commonly observed in infections with Type I
pneumococci   In particular, homologous type-specific antibodies were demon-
strated in patients from whom the following types were obtained   Types II,
III, V, VII, VIII, IX, XII, XIV, XVII, XVIII and XIX   Among the pa-
tients with Types II and V (IIa, Avery) pneumococci, homologous type-
specific antibodies were found for each type, with only occasional development
of heterologous antibodies as demonstrated by mouse protection tests   With
Types III and VIII (atypical III, Sugg and Harris) a similar specificity in
the production of antibodies occurred, but, in addition, there was some produc-
tion of Type III antibodies in pneumonias associated with Type VIII pneumo-
cocci and vice versa

The findings, in general, tend to confirm the biological identity of the newly
classified types and point toward their etiological relationship to human disease

Digestion Efficiency in Simple Undernutrition   By J M STRANG and H B
    McCLUGAGE (by invitation) and FRANK A EVANS, Pittsburgh, Pa

Nine undernourished patients increased their diets to a daily average of
3475 calories on which they gained an average of 7 3 kgm in 5 6 weeks   The
average weight of the food was 1926 grams per day   The average daily weight
of the stool was 165 grams or 8 5 per cent of the food intake   This ratio varied
according to the water content of food and stool   The food solids bore a
fairly constant relation to the stool solids from week to week   The average
weight of the food solids was 549 grams, of the stool solids, 31 grams   This
represents a digestion and absorption efficiency of 94 per cent   The nitrogen
in the stools averaged 1 3 gram per day, or 12 per cent of the food nitrogen

These studies indicate an ability on the part of patients with uncomplicated
undernutrition to digest and absorb sufficient food to gain rapidly

Congenital Heart Disease Complicating Pregnancy A Report of Sixteen Cases
    By BURTON E HAMILTON (by invitation) and ROBERT STERLING PALMER,
    Boston, Mass

Congenital heart disease is not a common complication of pregnancy   At
the Boston Lying-in Hospital in over 500 cases of organic heart disease, con-
genital heart disease comprises less than three per cent   It is found as a com-
plication of pregnancy once in 3000 patients   This very rarity, plus the fact
that the striking physical signs of congenital heart disease sometimes *impress*

the first observer unduly and lead him to make an unnecessarily grave prognosis and finally a practical clinical lesson which we have drawn from our experience, has prompted us to make this report We are presenting in addition to our sixteen cases the scattered reports of fifteen cases encountered in the literature

We feel justified in drawing the following conclusions

1 Coarctation of the aorta is to be considered in any case of unexplained persistent hypertension The prognosis for the mother when this condition complicates pregnancy is probably good The fetus may die in utero, possibly due to diminished circulation to the lower half of the body

2 Congenital complete heart block is not a serious complication of pregnancy

3 Patent ductus arteriosus is compatible with successful childbearing, only one in eleven cases having symptoms which could be considered at all serious We suspect that the same may be said of intraventricular septal defect if operative delivery and obstetrical manipulations in themselves likely to induce shock can be anticipated and avoided The danger probably lies in a reversal of the left right to a right left shunt brought about by a fall in systemic pressure

4 Experience with six pregnancies in one patient with preexisting right left shunt suggests that operative delivery or obstetrical procedures likely to induce hemorrhage or shock may cause a serious and alarming exacerbation of this shunt. However, compensatory mechanisms already present may make the danger less formidable in these cases

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Do the Thebesian Vessels Play Any Role in Supplying Blood to the Heart? By L N KATZ and (by invitation) KENNETH JOCHIM and ANNE BOHNING Chicago, Ill

Wearn s contention of the auxiliary role of the Thebesian vessels has recently been questioned We have reinvestigated the problem, using a system by which the cannulated coronary arteries and coronary sinus form a circuit distinct from the cardiac chambers and great vessels the only communication possible between the two circuits is by way of the Thebesian vessels Bismuth injected into the cardiac circuit appeared in the coronary circuit as evidenced by chemical analysis of the coronary sinus blood. Bacteria injected into the coronary circuit also appeared in the coronary sinus blood and were found histologically in the capillaries and coronary vessels The Thebesian vessels can therefore carry blood from the heart chambers into the coronary circuit but the amount carried is still undetermined

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The Calorigenic Action of Thyroglobulin and its Constituents By J LERMAN and W T SALTER Boston, Mass

It has previously been shown by us that thyroxin polypeptide is as active when given by mouth as when given parenterally, *and that its activity is proportional to its iodine content* when compared with crystalline thyroxin Using the metabolic response of untreated myxedema patients to thyroxin polypeptide given in dosage of one milligram by mouth daily as a standard we find in three assays of whole thyroid gland with an equivalent thyroxin content and in four assays of whole thyroid gland with an equivalent total organic iodine content that the calorigenic action of whole thyroid gland depends upon its total organic and not upon its thyroxin (active) iodine content.

In another study 20 patients with treated myxedema were given in succession thyroid gland preparations from different sources for periods of time sufficient to determine the maintenance level of metabolism for each patient

The results indicate that the ration of a preparation of thyroid which will maintain a definite level of metabolism in a given patient or change this level is dependent upon its total organic iodine and not its thyroxin (active) iodine content. Since diiodotyrosin (the soluble iodine fraction in iodothyroglobulin) is inactive calorigenically when given alone, there remains to be explained the mechanism by which it assumes calorigenic properties when linked with other amino-acids in iodothyroglobulin

One patient with myxedema was fed thyroglobulin obtained from hyperplastic glands, two others were fed thyroglobulin obtained from colloid glands The calorigenic response in each case was proportional to the total iodine content. Diiodotyrosin peptone, obtained by peptic digestion of thyroglobulin, was inactive in one patient, whereas thyroxin peptone obtained in this way was active in the same patient in proportion to its iodine content

The results thus far indicate that no calorigenic activity is lost in the isolation of thyroglobulin from whole thyroid gland and that diiodotyrosin loses its activity in the first stage of proteolytic digestion of thyroglobulin

The Elastic Properties of the Emphysematous Lung and Their Clinical Significance By RONALD V CHRISTIE, Montreal, Can

The elastic properties of the lung have been analysed in cases of emphysema by means of simultaneous tracings of the tidal air and interpleural pressure With inspiration and expiration the interpleural pressure is found to fluctuate around that of the atmosphere, and an almost complete loss of the pulmonary elasticity can be demonstrated These changes can also be shown by vital capacity tracings, the reserve air if taken alone being greater than that taken after the complemental air   The respiratory level also shows characteristic fluctuation

The significance of these findings with regard to the increase in lung volume, and the dyspnoea and orthopnoea in emphysema is discussed, the paradoxical movement of the diaphragm during expiration being emphasized and explained on what is shown to be an active expiratory effort of the extrinsic muscles of respiration   To prevent this paradoxical movement a tight abdominal binder has been used in the treatment of emphysema, with encouraging signs of symptomatic relief

The relationship of the loss of elasticity to unequal ventilation of the lungs in emphysema is also discussed

The Association of Acalcification of Dentine with Hypoparathyroidism in Rats and the Cure of Same with Parathormone, with some Correlated Observations in Man By FULLER ALBRIGHT and (by invitation) MOSES S STROCK, Boston, Mass

The first interrelationship to be demonstrated between the parathyroid glands and calcium metabolism was in 1911 by Erdheim   He showed that parathyroidectomy in the rat is followed by acalcification of that dentine which is formed subsequent to the operation   It has surprised some observers that acalcification should occur in hypoparathyroidism when decalcification is such an important part of hyperparathyroidism. The work of Erdheim has been repeated with similar results, and, with the aid of parathormone, strata of acalcified dentine followed by strata of calcified dentine have been produced at will Decalcification of the dentine was never produced   The data are in accordance with the view that metabolic processes can cause acalcification of dentine, but not decalcification   Confirmatory observations from the teeth of patients with hypo- and hyperparathyroidism are made

Observations upon the Refractory Period of the Auricular Extrasystole in the Mammalian Heart By E Cowles Andrus and (by invitation) Paul Padget, Baltimore, Md

The Production of Inflammatory Lesions in the Hearts of Animals Allergic to Hemolytic Streptococcus By Caroline C Bedell and Rawley M Penick (by invitation) and Benjamin M Baker, Jr, Baltimore, Md

Seegal, Seegal and Jost recently produced, by the intrapericardial injection of the homologous antigen an intense inflammatory reaction in the pericardium, heart and aorta of rabbits sensitized to egg white The present report has to do with similar observations upon animals made sensitive to a beta hemolytic streptococcus Twenty eight normal rabbits received, over a period of several weeks, 0 1 cc of a saline suspension of living organisms into the skin At varying intervals after the beginning of sensitization the pericardial sacs were exposed and 2 cc of vaccine prepared from the streptococcus employed were injected The animals were killed twenty-four hours to six days later and the hearts were examined both grossly and microscopically Twenty five of these showed varying degrees of pericardial and myocardial inflammatory reactions somewhat similar to the changes described by Seegal, Seegal and Jost One other had extensive changes confined to the pericardium only and the other two had but slight pericardial changes Experimental lesions were compared with the spontaneous lesions of thirty one normal rabbits Another control group was composed of twenty normal unsensitized rabbits which were subjected to the pericardial injections similar to those described above Many of these had minimal pericardial inflammatory reactions but in all but one the heart muscles were normal

The Action of Oxygen in Counteracting the Effects of Alcohol By Alvan L Barach, New York City, N Y

The ingestion of alcohol by a subject performing a standard exercise test resulted in a marked increase in the pulse rate respiratory rate and pulmonary ventilation above the response of the same subject without alcohol When oxygen was inhaled instead of air, there was a consistent reduction in the pulse rate, respiratory rate and pulmonary ventilation of the alcoholic subject. The inhalation of oxygen in the normal exercising individual also resulted in a lower pulse rate respiratory rate and pulmonary ventilation than that obtained when the exercise was conducted in air, but the percentage difference between the effect of oxygen in the alcoholic subject was considerably greater than in the non alcoholic subject, suggesting that the inhalation of oxygen tended specifically to counteract the handicapping influence of alcohol on muscular exercise

The psychological manifestations of alcoholism were slightly too moderately relieved or prevented by the inhalation of oxygen, but this varied considerably in individual subjects

The Simple Dialysate Nature of Edema Fluids Contrasted with the Specialized Composition of Cerebrospinal Fluid By Dorothy Rourke Gilligan and Marie C Volk (by invitation) and Herrman L Blumgart Boston Mass

The concentrations of certain electrolytes and non electrolytes in ascitic chest and subcutaneous edema fluid have been compared to the concentrations in both arterial and venous blood serum in twenty seven instances in patients with nephritis, cardiovascular disease, carcinoma and cirrhosis of the liver

The differences in the concentrations of the various electrolytes between the sera and fluids are governed by the differences in the protein contents, regard

less of the site of fluid formation or the underlying diseased state studied   The distribution, in terms of water content, of sodium, chloride, and bicarbonate between serum and the edema fluids is in close agreement with that expected for simple dialysates in accord with Donnan's membrane equilibrium law, when the source of the blood sample is considered   The average distribution ratios found were

$$\frac{(Na^+) \; edema \; fluids}{(Na^+) \; serum} = 0\,96, \quad \frac{(Cl^-) \; serum}{(Cl^-) \; edema \; fluids} = 0\,98, \; and$$

$$\frac{(HCO_3^-) \; serum}{(HCO_3^-) \; edema \; fluids} = 0\,96,$$

for serum representative of blood midway between arterial and venous   The theoretic Donnan ratio for this series was 0 955   These results indicate that no bicarbonate is "bound" in the serum   The distributions of calcium, potassium, and inorganic phosphate indicate that fractions of these substances are bound in the serum   The concentrations of the non-electrolytes, sugar, total nonprotein nitrogen and creatinin were practically equal in serum water and corresponding fluid water, in accord with a simple dialysate nature for these body fluids   The distribution of the substances between serum and the edema fluids corresponds to the distribution found by Greene et al (J Biol Chem, 1931, xci, 183) and others, between plasma and "in vivo" dialysates

Our investigations show that the edema fluids studied are simple dialysates in equilibrium with blood plasma   This finding supports the hypothesis that these fluids have collected in abnormal amounts by virtue of an imbalance of the normal forces of formation and reabsorption and is in harmony with the concept that edema is primarily a quantitative rather than a qualitative abnormality

A comparison of our studies with the spinal fluid measurements of other investigators has an important bearing on the theories regarding the mechanism of spinal fluid formation by the choroid plexuses, for it indicates that spinal fluid is not a simple dialysate   The concentrations, expressed in terms of water content, of sugar, creatinin, total nonprotein nitrogen, and inorganic phosphate, are much lower in the spinal fluid than in the serum, contrasted with practically equal concentration of these constituents in edema fluids and serum   The calcium content of spinal fluid is lower than the calcium content of edema fluid with similarly low protein content or than the "in vivo" dialysate   The difference in chloride concentration between serum and spinal fluid is much greater than the chloride difference between serum and the edema fluids or the "in vivo" dialysate, when the differences in protein concentrations between the sera and the two types of fluid are similar   Expressed in terms of water content, the ratio ((Cl⁻) serum/(Cl⁻) spinal fluid) is too low to accord with the theoretic Donnan ratio and conversely, the ratio ((HCO_3^-) serum/(HCO_3^-) spinal fluid) is too high   The choroid plexus must be considered a specialized structure and the fluid elaborated from it a specialized fluid rather than a simple dialysate

The Basal Respiratory Quotient and the Effect of a High Fat Meal on the Respiratory Quotient and Heat Production of Normal and Obese Individuals By BYRON D BOWEN and (by invitation) FRED R GRIFFITH, JR, and GRACE E SLY, Buffalo, N Y

An attempt to demonstrate a qualitative metabolic difference between obese and normal individuals was undertaken   All subjects had previously taken an

unrestricted diet and were given the same test meal (100 grams of butter and 50 grams of mayonnaise) The Tissot spirometer and the Haldane gas analyzer were used Tests not showing fairly steady ventilation volumes and CO_2 percentages in the expired air were rejected in order to eliminate the influence of hyperventilation The effect of the meal was observed for six to ten hours

The basal respiratory quotients of normal and obese subjects were 0 825 and 0 767—an average of 13 and 21 determinations (subjects) respectively A similar difference was observed by Hagedorn et al, although the values obtained by them were higher due to a diet which had been rich in carbohydrate The ingestion of the fat meal brought the quotients of both groups to approximately the same levels—about 0 80, indicating that both groups derived their extra energy for the specific dynamic action from the combustion of a similar mixture of carbohydrate and fat. This is opposed to the conclusions reached by Strouse and his coworkers from similar studies on a much smaller group

Similar studies made on a group of 11 obese diabetic patients at a time when the diabetes was under control by diet (but one had been taking insulin), showed the average respiratory quotient to be 0 765 The rise after the fat meal was less (0 782) than in the non diabetic obese group suggesting that the available carbohydrate reserve is less in the diabetic

The specific dynamic action was essentially the same for all groups, which opposes the idea that obese individuals are unique in this respect

Levels of Lactic Acid in Blood from Peripheral Vessels of Normal Subjects and Subjects with Claudication By GEORGE E BROWN and (by invitation) GRACE M ROTH, Rochester Minn

Levels of lactic acid sugar, oxygen content and saturation of the blood of the femoral and brachial arteries and femoral and basilic veins have been studied in normal subjects and in subjects with occlusive disease of the major arteries of the upper and lower extremities The relationship of levels of lactic acid and sugar has been investigated in relation to exercise and ischemic pains (claudication) The effect of cervicodorsal and lumbar sympathetic ganglionectomy on the lactic acid has been studied A correlation has been demonstrated between the levels of lactic acid in the venous blood and the excessive fatigue and pain of intermittent claudication The correlation is not sufficiently high however, to indicate a causal relationship between the two

Observations on the Circulation During and After Pregnancy By C SIDNEY BURWELL Nashville Tenn, and (by invitation) W DAVID STRAYHORN JR New York City N Y

Normal young women in the early months of pregnancy were selected and trained as subjects The acetylene method of Grollman was used to determine the cardiac output per minute it was applied under the usual standard conditions in regard to rest and food Observations were begun in the third or fourth month of pregnancy and continued at intervals of 2 to 6 weeks until delivery They were repeated during the puerperium in each case

In three of five women not only was there definite increase in cardiac output during pregnancy but this was greater than the increase in oxygen consumption so that the arteriovenous difference was decreased

In two patients there was no consistent elevation of cardiac output during pregnancy although isolated observations showed values above normal These patients had no unusual respiratory or circulatory symptoms or signs

In three women the cardiac output during the last weeks of pregnancy was lower than during several months preceding   In two of them it approached the values observed after delivery

In all five women the cardiac output and the A–V difference after delivery were not beyond the limits of normal

No striking variations in blood pressure were observed   The pulse was more rapid during than after pregnancy

Water and Sodium Chloride Balance in Patients Before and After Surgical Operations   By JOHN D STEWART and JOHN H TALBOTT (by invitation) and EDWARD D CHURCHILL, Boston, Mass

The water and salt balances of 16 patients were studied in several types of surgical operations   In 3 patients on whom a herniotomy was done no significant negative balance of sodium chloride was observed   The largest decrease in serum chloride was 3 0 m Eq , the average weight loss in the 5-day period of observation was 2 5 kgm

In 4 patients on whom a partial gastrectomy was performed the average negative balance of sodium chloride was 44 grams   The average decrease in serum chloride was 2 7 m Eq and the greatest weight loss was 1 7 kgm   In calculating the salt balance account was taken of chloride in vomitus and urine only   In the absence of diarrhea a small amount was presumed to be excreted in the stools   The remainder of the amount not accounted for was assumed to be lost in the sweat and drainage from the wound

In 2 patients, on whom three-stage thoracoplasties were done, interesting findings were the changes in serum chloride   Four days after the first stage operation there was a decrease of 12 4 and 18 7 m Eq in the respective patients   Following the second stage operation the greatest fall was 6 6 and 8 0 m Eq and following the third stage was 1 4 m Eq in the first patient   In the absence of noteworthy changes in the sodium chloride intake and output the above is taken as evidence of adjustment and adaptation to repeated loss of salt

Transformation and Dissociation of Pneumococcus   By M H DAWSON, New York City, N Y

Previous work on transformation of type-specific pneumococcus by *in vitro* procedures is briefly reviewed   Evidence is presented which suggests that S forms of one specific type may be directly transformed into S forms of other specific types without passing through the intermediate R phase

A new colony variant of pneumococcus is described   This new colony variant presents characteristics strikingly different from those of the previously described R and S forms   The morphology of the individual organisms constituting the new colony variant is also quite unique   Evidence is presented which suggests that the new phase of pneumococcus is an indirect and not a direct product of the parent form, from which it is derived   On the other hand reversion of the new phase to its parent form appears to be sudden and abrupt   The observations indicate that the evolution of the various phases may be cyclical in character

Bacterial dissociation in other species will be briefly discussed and the place of the new phase of pneumococcus in the general phenomenon of bacterial dissociation indicated   The evidence suggests that it will be necessary to revise the currently accepted terminology for dissociate forms of pneumococcus in accord with the following   (1) M (mucoid)—formerly S (smooth) , (2) S (smooth)—formerly R (rough) , (3) R (rough)—not previously described

Studies on Effective Barium Chloride Dosage   By ARTHUR C DE GRAFF and
(by invitation) JEAN A CURRAN New York City N Y

In 1909 Rothberger and Winterberg's fundamental researches established the basis for the present clinical use of barium chloride. It was found that small amounts injected intravenously in dogs prevented ventricular standstill after combined nerve stimulation Applying this finding to Adams Stokes' syndrome Cohn and Levine in 1925 prevented seizures of ventricular standstill with doses of 30 to 60 mgm three times a day On the other hand as much as 0 6 of a gram a day has been given without toxicity or benefit Because of this confusion as to dosage both therapeutic and toxic, the present investigation was undertaken first to ascertain if a definite end point of effective dosage could be determined second to determine the effective barium chloride dosage on dogs uninfluenced by any anesthetic, narcotic or operative preparation and third to compare dosage by vein and by mouth in both dog and man Since in any drug in which the end point of therapeutic effect is in doubt, it is desirable to increase the dosage to the point of mild toxicity, experiments were carried through to this level

It was found that in dogs 0 41 to 0 91 mgm injected intravenously per kilogram of body weight caused mild toxicity and unmistakable changes in the electrocardiogram namely ventricular premature contractions, frequently with coupling and changes in the character of the T wave When given by mouth 9 7 to 18 9 mgm were needed to achieve similar results Doses of over twice this amount caused moderately severe toxicity, but there were no fatalities In man 0 63 to 0 88 mgm per kilogram of body weight were needed intravenously to give a definite end point, while 3 7 to 13 2 mgm per kilogram had to be administered by mouth to get parallel results It was found that intravenously the greater the speed of injection the greater the effect Electrocardiographic changes caused by barium chloride given by mouth generally persisted as long as 6 to 7 hours but disappeared inside of 18 hours

Conclusions

1 The electrocardiogram reveals changes following barium chloride which are constant and furnish an end point as a definite guide in effective dosage This is probably above the therapeutic dosage and though mildly toxic is far below the lethal dose

2 The electrocardiogram furnishes a guide as to the rate of elimination

3 Using these end points fairly constant relationship exists between the kilogram body weight and effective intravenous dosage

4 For a given speed of injection there is a remarkably close comparison of effective dose by vein per kilogram of body weight between subjects both dog and man

5 Using electrocardiographic changes as our end point of dosage we have shown the variability of comparative effective dosage by mouth from one subject to another

An Aid in the Diagnosis of Subacute Bacterial Endocarditis By CLIFFORD
L DERICK and SAMUEL A LEVINE Boston Mass

Derick and Fulton in their paper on skin reactions of patients and normal individuals to protein extracts of streptococci reviewed the literature concerning the response of patients with subacute bacterial endocarditis to skin injections of streptococci and their products

Since that time we have continued these observations In all, 20 patients

with this disease have been studied   Thirteen of these showed a positive blood culture   The other 7 had all the clinical evidences necessary for this diagnosis

Nucleoprotein extracts of hemolytic, green and indifferent streptococci were used as test materials   Only one case in which the diagnosis was not confirmed by blood culture showed a mildly positive response to the hemolytic streptococcus nucleoprotein   In all the other instances the tests were negative   In addition, one patient was tested with 0 2 cc of a 24-hour broth culture of the autogenous viridans strain and another with approximately twenty million living organisms of *Streptococcus scarlatinae*   Both of these patients failed to show any reaction

All cases where subacute bacterial endocarditis was suspected on admission were tested as a routine procedure   Since none of the patients proven to have subacute bacterial endocarditis showed a reaction, the finding of a positive test practically rules out this diagnosis   We have found this test of considerable diagnostic value

The Effect of Arteriosclerosis and of Benign and Malignant Hypertension on the Area of Histamine Flares   By A CARLTON ERNSTENE and (by invitation) MAURICE SNYDER, Cleveland, Ohio

In 30 normal individuals between the ages of 18 and 58 years, the average area of the flare produced by injection of histamine into the skin of the forearm was 31 sq cm   The smallest flare observed had an area of 18 sq cm, while an area of less than 25 sq cm was observed in only 6 individuals   In a group of elderly subjects with advanced grades of arteriosclerosis but with normal arterial blood pressure, flares of reduced area usually were recorded, while in other patients with slight or moderate thickening of the arterial walls, little or no diminution was the rule   Flares of normal area were obtained in 21 individuals with essential hypertension of the benign type   In 11 subjects with malignant hypertension however, the average area of the flare was 14 sq cm and in only one patient did it exceed 19 sq cm   Only two of the patients presented an advanced grade of arteriosclerosis

Lewis demonstrated that the flare following a histamine stimulus results from dilatation of the strong arterioles of the skin   The diminution in the area of the flares observed in subjects with malignant hypertension and in those with advanced arteriosclerosis suggests the presence of structural changes in the walls of these vessels, although in malignant hypertension the possibility of severe and permanent vasoconstriction must be considered

The results of the investigation indicate that measurement of the size of the histamine flare affords data of considerable importance in the clinical study of patients with essential hypertension

The Relationship of Cerebrospinal Fluid Pressure to Systemic Blood Pressure   By FRANK FREMONT-SMITH and (by invitation) H HOUSTON MERRITT, Boston, Mass

A comparative study of systemic blood pressure and cerebrospinal fluid pressure has been made in over 1600 patients

In 1418 patients the systolic blood pressures ranged from 50 to 300 mm Hg and the diastolic blood pressures ranged from 30 to 190 mm Hg   A study of these cases showed no significant correlation between the level of blood pressure and the cerebrospinal fluid pressure

In 125 patients with elevated cerebrospinal fluid pressure, varying from 200 to over 1300 mm of water, the blood pressure was rarely elevated excepting when the cerebrospinal fluid pressure was extremely high, approaching the level of the normal diastolic blood pressure   In 22 cases with cerebrospinal

fluid pressure varying from 500 to 800 mm of water, only 4 had a systolic blood pressure of 150 mm Hg or over

In 63 patients with uremia or congestive heart failure, the cerebrospinal fluid pressure was frequently elevated but showed no correlation with the systemic blood pressure

These results indicate that in man, as has been previously shown in animals, the cerebrospinal fluid pressure is not directly related to the systemic blood pressure and that an elevation of the cerebrospinal fluid pressure has no appreciable effect on the systemic blood pressure until the cerebrospinal fluid pressure level approaches the normal level of the diastolic blood pressure When this occurs the systemic blood pressure becomes elevated to maintain cerebral circulation A short discussion of the physiological mechanisms involved is given

Conclusions

1 The level of systolic or diastolic blood pressure has no appreciable influence upon the cerebrospinal fluid pressure

2 In uremia and congestive heart failure the cerebrospinal fluid pressure is frequently elevated

3 The level of cerebrospinal fluid pressure has no appreciable effect on systemic blood pressure unless the cerebrospinal fluid pressure is greatly increased so as to approach the level of the diastolic blood pressure Under these circumstances there is a rise in the systemic blood pressure

The Interpretation of the Blood Sedimentation Rate (With Reference to Curves Obtained with Special Recording Apparatus) By A PROSKOURIAKOFF (by invitation) and BURGESS GORDON, Philadelphia Pa

Comparisons are made between curves obtained with the usual technique of interval charting and those with a special electrical recorder which plots continuously the level of sedimentation The data suggest that variations in the degree and rate at certain periods may be significant in the interpretation of the curves

A Critical Examination of Methods for Determining the Cardiac Output in Patients with Cardiac Disease By T R HARRISON and (by invitation) ARTHUR GROLLMAN, BEN FRIEDMAN, and GURNEY CLARK, Nashville, Tenn

The accuracy and applicability of the acetylene method, the Burwell Robinson method and the venous plateau method have been studied by comparing them with each other and by testing the validity of the assumptions underlying them In normal subjects and in certain persons with mild congestive heart failure the two latter methods agree excellently with the acetylene method which is based on an entirely different principle However the three methods as ordinarily applied disagree in certain patients with heart failure and these have been the object of special studies from which the following conclusions are drawn

1 In certain cases with heart failure the Burwell Robinson method may occasionally yield inaccurate results and therefore cannot be used with safety

2 The venous plateau method gives accurate results when certain rigid criteria are adopted For technical reasons and because of the discomfort to the subject involved in the procedure it is not usually the method of choice

3 The acetylene method may yield false results when only one determination of the arteriovenous difference is made at a single rebreathing When two

measurements are made of the arteriovenous difference during a single rebreath-
ing and are found to agree, the results are accurate   Because of its technical
advantages this acetylene plateau method is the procedure of choice
    Criteria have been described whereby in a given case the applicability of a
given method may be tested, so as to have no doubt of a given result   Unless
these criteria are rigidly adhered to, markedly erroneous results may be ob-
tained   In patients with severe congestive heart failure these criteria may
be impossible of fulfillment.

*The Interference with the Pulmonary Capillary Circulation by Fat and the Ef-
    fect of Intravenous Alcohol Dextrose*   By LOUIS G HERRMANN (by
    invitation) and GEORGE R HERRMANN, Galveston, Texas
    Rabbits and dogs were carefully selected and in double pairs injected at a
uniform speed and without undue force at the rate of 1 cc per minute with
warm (37° C) liquid, sterile, neutral fat in doses of 1 cc per kilo of body
weight   One animal of each series was kept as a control   Manifestations of
disturbances of pulmonary circulation and the resulting anoxemia, as extreme
restlessness, severe dyspnea and cyanosis, appeared rather promptly   The
symptoms consistently disappeared almost immediately following the intra-
venous injection of 5 cc per kilo of body weight of the alcohol-dextrose solu-
tion   This was repeated at the end of 12 hours in two of the series and in
24 hours in one of the remaining two   At the end of 48 hours all animals
were sacrificed and the lungs promptly and completely fixed showed a sharp
decrease in the amount of neutral fat globules in the pulmonary capillaries
according to the number of injections with almost a complete clearing in those
animals that had received three injections
    Data are being accumulated on the types and amounts of neutral fat, fatty
acids, cholesterol and phosphatids by volumetric determination in the arterial
and venous blood, in the general and in the pulmonary circulation, before and
after the injection of neutral fat, and after the injections of alcohol-dextrose
The quantitative distribution and the rate of disappearance of fat from the
blood stream have been determined

Clinical Manifestations of Hypo- and Hyper-Magnesaemia   By ARTHUR D
    HIRSCHFELDER, Minneapolis, Minn
    Although blood magnesium is usually constant (2 to 3 mgm Mg per 100 cc)
in normal individuals, we have observed two distinct clinical groups in which
significant alterations of blood magnesium were associated with concomitant
clinical manifestations
    1 Low blood magnesium (0 9 to 1 37 mgm) was found in 10 cases, all
manifesting twitching or convulsions   One of these was parathyroid tetany,
two epilepsy, one cerebral injury, the others acute or chronic nephritis   Three
of the latter were given Epsom salts by mouth, whereupon the blood magnesium
rose to from 3 3 to 5 8 mgm Mg, and twitchings or convulsions disappeared
    There thus seems to be a definite clinical syndrome associated with low blood
magnesium
    2 High blood magnesium   This was found especially in nephritics after
the administration of Epsom salts   In normal individuals 20 to 30 grams
$MgSO_4$ $7H_2O$ by mouth does not significantly raise the blood magnesium and
about 40 per cent is excreted in the urine in 24 hours   In nephritics less of
the ingested Mg is excreted in the urine, and the blood magnesium rises
    Twenty to 30 grams $MgSO_4$ $7H_2O$ administered to 9 patients with acute

and chronic glomerulonephritis raised blood magnesium from near normal to 9 8 to 11 3 mgm All these patients were definitely more drowsy when at these levels Two nephritics with blood magnesium 8 9 to 9 0 mgm without administration of $MgSO_4$ were definitely drowsy and unresponsive.

Coma is induced in animals when the blood magnesium approaches 17 mgm Mg per 100 cc

Elevation of blood magnesium to about two thirds of the coma level thus seems to be accompanied by a tendency to somnolence

It is probable that repeated purgative doses of Epsom salts by mouth can induce coma in nephritics, and that many cases of supposed uremic coma are really magnesium coma induced by Epsom salts From this coma animals and probably patients can be awakened and their lives prolonged by intravenous calcium chloride

Nephritic rabbits whose blood magnesium is moderately elevated (5 to 10 mgm Mg) by orally administered $MgSO_4$ are hypersensitive to morphine

The Blood Pressure in Stenosis of the Isthmus (Coarctation) of the Aorta By JOHN T KING, Baltimore, Md

The blood pressure in the arms in cases of isthmus stenosis (coarctation) of the aorta is so often greater than normal that many observers have assumed hypertension to be a necessary part of the diagnostic picture In the author s experience hypertension was found in the arms in ten of eleven cases One patient showed typical signs of coarctation including relative hypotension in the legs, but in her case the arm pressures were always within normal limits

An analysis of the literature showed normal arm pressures present in twenty per cent of cases of isthmus stenosis in which pressure readings were recorded The author's case is the seventeenth reported instance of normal arm pressure in coarctation of the aorta

The Effects of Alternate Suction and Pressure on Blood Flow in the Lower Extremities of Normal Subjects and of Patients with Peripheral Vascular Disease By EUGENE M LANDIS and (by invitation) JOHN H GIBBON, JR Philadelphia, Pa

The lower extremities of normal subjects and of patients with peripheral vascular disease were inserted into an aluminium box through a well fitted, heavy rubber diaphragm Negative pressure, amounting to 120 mm Hg was applied for 25 seconds alternating with positive pressure amounting to 100 mm Hg for 5 seconds

Increase in blood flow to the distal portion of the lower extremity was identified by thermo electric measurements of digital skin temperatures In normal subjects the extremity exposed to alternate suction and pressure cooled more slowly than the control extremity When vasoconstrictor tone was diminished slightly by immersing one forearm in warm water the temperature of the extremity exposed to suction and pressure rose earlier, and to a greater height than that of the control extremity With the subject chilly and the extremities cold suction and pressure in some observations had no measurable effect on blood flow until vasoconstrictor tone was slightly diminished by warming the body In most instances the control extremity cooled while the rising temperature in the extremity exposed to suction and pressure indicated an increase in blood flow on that side

In patients with peripheral vascular disease alternate suction and pressure was accompanied by conspicuous warming (and therefore by increased blood

flow) in extremities showing no rise in temperature during anesthesia of the posterior tibial nerve   In the presence of obstructive lesions of more moderate grade the more involved (and cooler) extremity was made the warmer of the two extremities by alternate suction and pressure

The results indicate that this apparatus may possibly be used as a " peripheral heart " to increase blood flow in the lower extremities of patients with peripheral vascular disease.

The Normal White Blood Cell Picture   By Edgar Jones and Doran J Stephens (by invitation) and John S Lawrence, Rochester, N Y

Data have been collected from observations on sixteen normal adults which indicate that there are slight variations in the number of the white blood cells in the peripheral circulation under basal conditions   These fluctuations have been found to be greater than our error of technique   Determinations of the number of white blood cells have been made at 15 minute intervals over four hour periods   Differential counts have shown that all of the cells show fluctuations but the greatest of these are shown by the neutrophils   No rhythm, no showers of " non-motile " cells and no appreciable variations in the number of " stab " forms have been found

The effect of change of posture has been carefully investigated in thirty-five normal adults and eighteen adults with a variety of clinical disorders   No significant variation has been found to be associated with change in position

Observations on the effect of digestion on the white blood cell count in thirteen normal adults have shown a slight increase in the number of the cells in the majority of these individuals two to four hours after the ingestion of a large meal   The neutrophils account for most of the changes in the total number of white blood cells in these subjects

Observations of the Cerebral Blood Flow in Man   By F A Gibbs and E L Gibbs (by invitation) and W G Lennox, Boston, Mass

A thermo-electric blood flow recorder has been devised for measuring the rate of blood flow through the internal jugular vein of man   Continuous records have been made over a period of three or four hours of the variations in flow through the internal jugular vein of unanesthetized human subjects   Records have been obtained of the flow associated with epileptic convulsions and with sleep   Observations so far fail to show significant changes in cerebral blood flow immediately preceding these events   The effect on cerebral blood flow of alterations in blood gases and of the administration of certain drugs has been studied   High CO_2 and low O_2 increase and low CO_2 decreases cerebral blood flow   Adrenalin produces a marked increase in flow, as do also amyl nitrite and acetyl cholin unless the fall in systemic blood pressure is too great   Caffeine and ergotamine tartrate produces a more lasting increase in cerebral blood flow than any of the other agents studied

Single Cell Inoculations with Treponema Pallidum   By Clarence S Thomas (by invitation) and Hugh J Morgan, Nashville, Tenn

The development of syphilis in the rabbit following intratesticular injection of test material affords positive evidence of the presence of *Treponema pallidum* in the inoculum   Failure to thus infect has been interpreted as indicating the absence of *Treponema pallidum* in the inoculum   Inasmuch as this interpretation is widely used in the study of experimental syphilis (immunity, chemotherapy) it seemed desirable to test the validity of the assumption upon which it is based

In preliminary experiments inocula, in which the presence of a few organisms seemed probable, failed to produce infection An attempt was made to definitely determine this point by the inoculation of single organisms A drop of diluted syphilitic testicular emulsion was placed in a moist chamber By dark field illumination and with a micro manipulator it was possible to aspirate one or several organisms into fine glass pipettes The pipette tips were immediately placed in syringe needles and injected into the testicles of rabbits The testicles were then vigorously massaged

Eighteen rabbits were thus injected with single organisms (11 Nichols strain 7 'S'' strain) Four additional animals were inoculated, three with two organisms each (Nichols strain) and one with six organisms ('S'' strain) All remained normal At appropriate intervals transfers to a second series and from the second to a third series of rabbits were made all with negative results Immunity to the homologous strain was not present in those animals of the third series which were re inoculated

The Cholesterol Content of the Blood in Obesity Including Observations of the Effect of a Diet Low in Fats Carbohydrates and Total Calories on the Blood Cholesterol[1] By MAURICE BRUGER[2] (introduced by Herman O Mosenthal) New York City, N Y

Two hundred and seventy seven plasma cholesterol determinations were made on seventy five cases of obesity The percentage of overweight varied from 20 to 122 per cent, with an average of 47 7 per cent for the group

Uncomplicated (frank exogenous) obesity is associated with a normal plasma cholesterol Diabetes mellitus hypertension, arteriosclerosis and hypo thyroidism tend to elevate the plasma cholesterol in the obese

The normal plasma cholesterol in obesity is not affected materially by low-caloric, low fat diets in patients followed at regular intervals for approximately one year The hypercholesterolemia in obesity complicated by diabetes mellitus, etc usually shows a distinct elevation the first few weeks on low caloric, low-fat diets but tends to fall appreciably below the control level following several months on such a regime

General considerations of diet and loss in weight in the obese in relation to the plasma cholesterol are discussed

Leukocytosis Following the Intramuscular Injection of Liver Extract By WILLIAM P MURPHY and (by invitation) JOHN H POWERS and KATHA RINE HUMPHREYS Boston Mass, and Cooperstown N Y

Determinations of the total white cells and the total number and percentage of polymorphonuclear neutrophiles in the peripheral circulating blood of 16 normal subjects before and after the intramuscular injection of liver extract have been presented in graphic form The individuals were divided into 2 groups ambulatory and recumbent

The average maximal increase in the total number of white cells of the ambulatory group was 94 1 per cent higher than the average of all control counts and occurred 7 hours after the extract was administered The greatest individual response in this group was 185 per cent and the lowest was 23 per cent above the average of the 4 control counts made on each of these 2 subjects the previous day The average maximal increase in total white cells of the patients of the recumbent group was 72 per cent above the normal level and

flow) in extremities showing no rise in temperature during anesthesia of the posterior tibial nerve   In the presence of obstructive lesions of more moderate grade the more involved (and cooler) extremity was made the warmer of the two extremities by alternate suction and pressure

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water bath for 1 hour, the difference in O_2 content, minus that used by the blood alone, was taken as the amount consumed by the tissue Liver and kidney tissues from 15 anemic and 15 control rabbits were used, the iron content of tissue and blood serum being determined

Under these conditions tissues from anemic animals do not utilize as much oxygen as those of normal tissues by about 50 per cent The results demonstrate the fact that, in general, anemia affects all the tissues and not the blood alone

The Excretion of Bence Jones Protein in the Nephrotic Syndrome By Howard F Root and (by invitation) Hazel M Hunt, Boston, Mass

A case is presented of a 12 year old boy showing edema albuminuria, low serum protein content inversion of the albumin globulin ratio in blood serum and the hypercholesterolemia characteristic of nephrosis and excreting considerable amounts of albumin and of albumose of the Bence Jones type The 24 hour excretion of albumin varied from a trace to 9 5 grams The albumose varied in amount but it was possible once to isolate as much as 1 5 gram from a 24 hour specimen in a finely powdered condition by precipitation at 55° C This isolated material gave the characteristic tests for Bence-Jones protein The use of a high protein diet (protein 130 to 160 grams, carbohydrate 160 grams, and fat 100 to 150 grams) and thyroid extract (gr v daily) brought about the following results In a period of 4 months anasarca disappeared basal metabolism rose from — 16 to + 7 per cent serum protein rose from 2 9 to 4 7 per cent. The albumin globulin ratio returned to normal The excretion of Bence-Jones protein in the urine ceased The plasma cholesterol rose steadily from 439 to 1250 mgm per 100 cc. A constant retention of nitrogen averaging 10 grams daily occurred during the first 6 weeks Nitrogen balance was reached in 8 weeks No cause other than the nephrotic syndrome was found for the occurrence of the Bence-Jones protein

Even when the albumosuria was most marked no albumose was found in the blood plasma This fact suggests that abnormality in the renal mechanism was responsible for the albumosuria rather than the formation of albumose in other tissues transportation by the blood to the kidney and its excretion

It has been held that the hypercholesterolemia of nephrosis is due to tissue starvation The degree of tissue inanition in this case may be judged by the fact that for 6 weeks he retained nitrogen in amounts varying from 6 to 12 grams daily The plasma cholesterol of 1250 mgm. is higher than any figure mentioned in Leiter s review of the subject As yet the cholesterol has not begun to decrease although the clinical improvement is most marked

The disappearance of edema when the serum protein remained at a low level indicates that some additional mechanism was involved in maintaining the osmotic pressure within the vessels It is suggested that the high concentration of cholesterol acted in this manner to prevent edema

Studies on Specificity of Streptococci The Experimental Reproduction of Persistent Sneezing with a Streptococcus Isolated from a Case By Edward C Rosenow, Rochester, Minn

The streptococcus isolated from the nasopharynx of the patient on the eighth day of persistent sneezing and from the brain and other tissues of inoculated animals was grown in dextrose brain broth and the cataphoretic velocity was determined in distilled water with the Northrop Kunitz Mudd apparatus

Rabbits were inoculated intracerebrally intratracheally and intraperitoneally, and dogs intracerebrally Persistent sneezing, which continued to recur often

occurred 6 hours after the injection of liver extract   The highest and lowest individual responses in the members of this group were 101 and 20 per cent

A similar increase in the total number of white cells and the total number and percentage of polymorphonuclear neutrophiles was obtained in one patient with influenza

The leukocytosis in every instance was due to an increase in the polymorphonuclear neutrophiles

The Occurrence of Pneumococcus Variants in Lobar Pneumonia   By JOHN R PAUL and (by invitation) J A LAWRENCE, New Haven, Conn

Whether or not S pneumococci dissociate into avirulent variants during the clinical course of lobar pneumonia is the subject of this report   The work has been based upon recent observations upon the *in vitro* dissociation of S pneumococci which show that beside the well recognized and easily revertible, intermediate and R variants, there are also so-called R G variants, some of which are bile insoluble and apparently indistinguishable from *Streptococcus viridans* These are difficult to revert back to the S forms

Twenty-seven cases of lobar pneumonia were studied with daily throat cultures upon which differential bacterial colony counts were made   A shift in the throat flora was found at or about the time of crisis, S forms diminish, and suspected, unstable R and R G forms appear in great numbers during the first few postcritical days   To establish the identity of these suspected R G forms the attempt to revert strains isolated before and after crisis was made in about twenty-five instances   Three successful reversion experiments are reported upon R G strains isolated in the first week after crisis   The results suggest that virulent pneumococci undergo marked dissociation within the human host during pneumonia   The clinical implications are briefly discussed

Observations on the Living Choroid Plexus   By TRACY J PUTNAM (Boston) and (by invitation) ERIC ASK-UPMARK (Lund, Sweden)

It is possible to observe the living choroid plexus of the cat under the microscope   By micrometry, the arteries can be seen to contract and expand under nervous and chemical influences, much as do the pial vessels   If the surface is stained with methylene blue, the choroid ependyma, the muscular coat of the arteries and occasionally nerve fibers may be demonstrated   The axones of the subjacent white matter are easily stained   If fluoresceine be injected intravenously, it may be seen to escape through the walls of the capillaries and veins, particularly the latter   This is perhaps of significance in relation to the pathogenesis of some cerebral diseases in which the lesions have a perivascular distribution

Tissue Metabolism in Secondary Anemia   By HERMAN H RIECKER, Ann Arbor, Mich

The object of this experiment was to determine whether the tissues of animals made anemic by hemorrhage could utilize oxygen at the same rate as those of normal animals   Previous work by Anson and Mirsky, Keilen, and Warburg indicated the possibilities that the iron-containing respiratory pigment (hem) of the cell might be partially depleted by iron starvation

A simple method was devised which seemed suitable for the study   Normal defibrinated rabbit's blood was oxygenated and 1 to 2 grams of the tissue macerated and mixed with the blood   Twenty cc of the blood was used and the oxygen content determined before and after exposure to the tissue in a

water bath for 1 hour the difference in O content, minus that used by the blood alone, was taken as the amount consumed by the tissue Liver and kidney tissues from 15 anemic and 15 control rabbits were used, the iron content of tissue and blood serum being determined

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for many hours, was produced by the different methods of injection of the live streptococcus as isolated and after many rapidly repeated subcultures in dextrose-brain broth, of suspensions of the heat-killed streptococcus and of filtrates of active cultures

The lesions produced were typical of encephalitis and were mainly in the medulla

The results in control animals injected in like manner with streptococci from patients having other diseases of the nervous system, were very different, in that sneezing was almost wholly absent and in that many animals revealed symptoms and other findings characteristic of the respective diseases studied

The cataphoretic velocity of the streptococci as isolated at the time of the attack or shortly after in the case of persistent sneezing, and in the control group, before and after animal passage, was mainly neurotropic   The neurotropic velocity and concomitantly the property of producing sneezing, were lost promptly on ordinary aerobic cultivation

A Significant Contrast Between Vasomotor Responses of the Peripheral Arteries   By W J MERLE SCOTT and JOHN J MORTON, Rochester, N Y

To determine the respective contributions of vasoconstriction and of mechanical occlusion has become essential in the clinical analysis of arterial diseases in the extremities   For this purpose two different vasomotor responses of the peripheral circulation have been used, viz (1) the effect of vasoconstrictor paralysis and (2) that of vasodilator stimulation, the tacit assumption being made that the two resultant effects are approximately identical   In a group of fifteen individuals with normal blood vessels this is found usually to be the case, both paralysis of sympathetic vasoconstriction and stimulation of sympathetic vasodilatation causing an elevation of the surface temperature in the foot to the " normal vasodilatation level "   Significant differences in the response of the local circulation to these two vasomotor reactions are found both in some individuals with normal and in those with pathological arteries   Occasionally the response to vasodilator stimulation is so much delayed or so much diminished as compared to the response to vasoconstrictor paralysis that the former is entirely unreliable for clinical purpose   As a corollary of this finding, in the clinical test unless an approximately maximal response to vasodilator stimulation is obtained the effect of vasoconstrictor paralysis should be ascertained

On the Diagnosis of Myocardial Disease by Means of the Basal Cardiac Work   By ISAAC STARR, JR, and (by invitation) J S DONAL, L H COLLINS, JR, A MARGOLIES, and C J GAMBLE, Philadelphia, Pa

The normal standards for basal cardiac work (cardiac output multiplied by mean blood pressure) have been determined by Starr, Collins and Wood (J Clin Invest, 1933, XII, 13)   Since that investigation the basal cardiac work and the heart size have been determined for about 100 additional cases, including all the types of heart disease that could be secured   In this presentation it is proposed to discuss these results from the clinical point of view

All cases in, or obviously threatened with, congestive failure were found to have myocardial insufficiency, i e. their hearts were doing abnormally small amounts of work per unit of heart size   Therefore these estimations may appear to be of value in the diagnosis of this condition   A considerable number of cases of hyperthyroidism and hypertension showed the same abnormality

On the other hand the majority of cases of angina pectoris and coronary

occlusion (an old infarct was later demonstrated at autopsy in two patients) had a normal basal cardiac work

Cases of effort syndrome show a small basal cardiac work, but give no evidence of myocardial insufficiency because they have small hearts and the work per unit of heart size is within normal limits

Alterations in the Specific Gravity of the Plasma of the Blood with the Onset of Diuresis in Patients Suffering from Heart Failure of the Congestive Type   By HAROLD J STEWART New York City, N Y

The mechanism of the occurrence and elimination of edema in heart disease is still incompletely understood   Where, in a system regarded as circulating blood located between the tissues and the kidneys, must a stimulus be applied in order that diuresis may be initiated?   Different types of diuretics may obviously act at different points   If the kidneys are first affected so that fluid passes from the circulating blood *concentration* of the blood should occur   If, on the other hand, the process is initiated in the tissues, fluid first enters the circulating blood and *dilution* occurs and may be detected if it lasts long enough and is great enough

Light on what the occurrences are when edema disappears may be gained by studying the blood to see whether concentration or dilution takes place   Changes in the specific gravity of the blood plasma may be utilized to trace the alterations in the blood   The method of Van Slyke and Moore has been used   It is now known that the specific gravity of the plasma of normal individuals varies from day to day within narrow limits only   It is moreover closely correlated with the amount of protein present in the blood   In cardiac patients the occurrence of edema does not depend on the level of specific gravity of the plasma which has in fact a normal value   We have investigated the curve of specific gravity with the onset and continuance of diuresis in patients suffering from heart failure of the congestive type who exhibited edema and accumulations of fluid   One case will serve to illustrate the alterations which were observed   B McL, female, white 56 years of age suffering from a second attack of heart failure of the congestive type, exhibited edema of the extremities   Arterial hypertension and enlargement of the heart were present. Rapid ventricular rate due to auricular fibrillation was present   Although the intake of fluids was restricted to 1200 cc. per day and she remained in bed the output of urine did not rise beyond 200 to 300 cc per day and she did not lose weight   the specific gravity of the plasma of the blood measured 1 0278   Digitalis 1 2 gram was given within 24 hours, the ventricular rate became slower diuresis occurred and the patient lost weight   The specific gravity of the plasma fell to 1 0240 and remained in that zone during diuresis but when diuresis stopped the specific gravity returned to the level it had had beforehand (1 0280)   Diuresis sometimes was accompanied by similar changes when it occurred spontaneously as a result of limitation in the administration of fluids and of rest in bed and also as a result of giving theocalcin   By taking samples of blood frequently it was found that decrease in the specific gravity of plasma appeared to precede the onset of diuresis   and that it was possible to predict the occurrence of diuresis   Estimation of the total amount of protein excreted by way of the urine during the periods of diuresis failed to account for the low concentration of protein indicated by the alteration in specific gravity   The decrease was due not to loss of protein from the blood, but to dilution of the blood by edema fluid   In another patient, in whom the specific gravity of the plasma of the blood before the onset of diuresis was 1 0270 the s₁ of the edema fluid was 1 0084   With the occurrence of d

gravity of the plasma fell to 1 0240, had approximately 400 cc of edema fluid having a specific gravity of 1 0084 entered the blood, the amount would have been sufficient to reduce the specific gravity of the plasma to the observed level (1 0240)

Using alteration in specific gravity as a measure of concentration of protein in the blood, it appears that diuresis, in the presence of heart failure of the congestive type, depends on changes initiated in the tissues, and is accompanied by dilution of the circulating blood

Constitutional, Hereditary and Familial Features of Pernicious Anemia By CYRUS C STURGIS and RAPHAEL ISAACS and (by invitation) L R GATES, Ann Arbor, Mich

A study of 680 consecutive patients with pernicious anemia and a similar number of non-pernicious anemia patients of the same locality and age groups was made, correlating the weight, hair color, eye color, ear length, blood pressure, spleen size, blood count, blood bilirubin, lymph node size, heart size, heart murmurs, gallbladder, age incidence, duration and individual symptoms   The disease incidence in the family and system incidence in the patients were correlated and the significance or non-significance was determined from Fisher's table of χ^2 A similar study was made, correlating the various symptoms of the patients   Pernicious anemia occurred more frequently in the families of the pernicious anemia patients than in the others   No significant correlation was evident between the special system disease tendency in any individual patient and a history of similar system disease in the family, although there was a moderate tendency for the association of these symptoms in the presence of a family history of heart disease, gastro-intestinal, genito-urinary, and skin disease and tuberculosis

The Absorption of Thyroxine from the Gastro-Intestinal Tract, with Special Reference to the Effect of Alkali By WILLARD O THOMPSON and (by invitation) PHEBE K THOMPSON, SAMUEL G TAYLOR, III, and LOIS F N DICKIE, Chicago, Ill

A large number of observations on twelve patients with myxedema show that pure synthetic thyroxine when given by mouth or directly into the duodenum has only a slight effect on the basal metabolism, whereas, when given by mouth in the form of its monosodium salt, it has about one-quarter to one-fifth as much effect as when given intravenously, and when given in solution with an excess of sodium hydroxide, it has a much greater effect which nearly equals its effect by the intravenous route   Dissolving thyroxine in an excess of sodium hydroxide results in the formation of the disodium salt   Squibb's thyroxine for oral use, which presumably contains a sodium salt of thyroxine and some thyroxine in peptide combination, has about two-thirds to three-fifths as much effect as thyroxine intravenously and about three times as much effect as the monosodium salt of synthetic thyroxine given orally

Since pure thyroxine is insoluble in most solvents and its monosodium salt only slightly soluble, while the disodium salt and thyroxine in peptide combination are much more soluble, it would appear that the solubility of the thyroxine compound administered is an important factor in its absorption from the gastrointestinal tract and hence in its effect on the basal metabolism   The solubility of thyroxine peptide and polypeptide derived from desiccated thyroid during digestion in the gastro-intestinal tract may explain the fact that desiccated thyroid by mouth has about the same effect as thyroxine intravenously on the basis of equivalent iodine contents

The Dissolution of Clotted Plasma by Hemolytic Streptococci the Relation of This Phenomenon to Acute Hemolytic Streptococcus Infections By WILLIAM S TILLETT and (by invitation) R L GARNER Baltimore Md

Hemolytic streptococci isolated from patients are capable of rapidly liquefying clotted human plasma   Sterile Berkefeld filtrates of full grown broth cultures contain the fibrinolytic property to a high degree   Broth cultures and sterile filtrates of hemolytic streptococci (beta type) derived from human cases possess this property when tested with clotted human plasma   The human strains fail completely to liquefy the fibrin clot of plasma derived from many animals   Available strains of hemolytic streptococci derived from animals are incapable of liquefying human clot   Fibrinogen, chemically isolated from plasma, when coagulated with thrombin in the presence of suitable culture or filtrate is rapidly liquefied   Dissolution under these conditions occurs with both animal and human material   The rate of liquefaction is such that 0 5 cc of culture or filtrate transforms 1 cc of solid clotted plasma into a completely fluid state within fifteen to twenty minutes   A comparable amount of fibrinogen, when coagulated in the presence of culture, is completely liquefied in five min utes

Pathogenic bacteria, such as pneumococci, green streptococci, typhoid bacilli, etc , similarly tested, have not exhibited a comparable fibrinolytic property

The fibrin digesting power of hemolytic streptococci suggests an explanation for the manifestations of acute streptococcus infections which are characterized by thin fibrin poor exudates such as are seen in the early stages of hemolytic streptococcus empyema

By repeated tests on plasma obtained from patients ill with hemolytic streptococcus infections, recovery frequently parallels or is followed by the development in the blood of a property by which clot digestion is inhibited   Effective resistance to the activity of the fibrinolytic ferment is most commonly demonstrable in the plasma of patients recovered from suppurative hemolytic streptococcus infections   The development in the blood of recovered patients of the capacity to inhibit this bacterial activity seems to be a form of resistance which differs in the parallel tests so far performed from streptococcal agglutinins or the anti streptolysin described by Todd

Observations on the Etiological Relationship of Severe Alcoholism to Pellagra By TOM D SPIES and H F DEWOLF (introduced by Joseph T Wearn), Cleveland, Ohio

It is generally agreed that the pellagra found in the North is usually associated with severe alcoholism   This study demonstrates that over ninety per cent of the patients develop the lesions of pellagra following excessive drinking   Ten patients having typical   alcoholic pellagra " recovered from their disease while receiving an adequate diet and large quantities of corn whiskey On theoretical grounds it may be considered that the whiskey predisposes to the development of pellagra either by destroying the so-called " pellagra preventive ' factor or by altering the gastro-intestinal tract so that it becomes incapable of assimilating the preventive substance   Likewise it has been suggested on theoretical grounds that foreign substances in the whiskey such as the higher alcohols produce the lesions of pellagra

It appears unlikely however that there is any specific deleterious material present in the many kinds of beverages taken   The present observations show that the consumption of large quantities of whiskey at the same time that an adequate diet is received does not interfere with the clinical improvement of

the patients   This is strong evidence that alcohol does not directly inactivate either all of the gastric secretion or all of the potent substances in the food   It is well known that many individuals drink large quantities of alcoholic beverages for years without suffering from loss of appetite, vomiting or pellagra   Correlation of these findings suggests the following hypothesis   Severe alcoholic imbibition by some individuals causes inadequate ingestion of food by decreasing the appetite and often precipitates nausea and vomiting   These predispose to the development of pellagra because the person no longer receives an adequate diet but utilizes the calories in the alcohol

The Clinical Significance of the Hyperactive Carotid Sinus Reflex   By SOMA WEISS and (by invitation) JAMES P BAKER, Boston, Mass
A study has been made of the circulatory and nervous system of 12 subjects who complained of dizziness and fainting, and in whom the mechanical stimulation of one or each of the carotid sinuses induced characteristic aura, dizziness, fainting and convulsive seizures   Three main types of cardiovascular responses were observed following stimulation of the sinus   (a) marked asystole or sudden slowing of the heart rate with or without marked fall in the arterial blood pressure, (b) marked fall in the arterial blood pressure, but without essential cardiac slowing, (c) changes in the cerebral circulation without essential slowing of the heart rate, and without fall in the arterial blood pressure   Stimulation of the hyperactive carotid sinus reflex induced striking changes in the intracardiac conductive system   partial and complete heart block, temporary asystole of the ventricle with continued auricular contraction, nodal rhythm, ventricular extrasystoles, change in the shape of the T waves, and complete inversion of the electrical axis in the heart   The observations also demonstrate that Adams-Stokes syndrome can be induced reflexly
Evidence is presented that the clinical symptoms and signs observed, as well as the cardiovascular changes, are due to stimulation of the sinus and not to direct motor vagal stimulation
During stimulation of the reflex, the volume and velocity of blood flow became decreased   There was also a slowing of the blood flow through the brain   In the precipitation of fainting and convulsions the rate (time element), rather than the absolute deviation from the normal circulatory state of the brain, plays the primary role   This temporary, sudden ischemia, even if of short duration, sets up a sequence of events in the brain which proceeds independently to convulsions even if hyperemia promptly follows the ischemia
Section of the carotid sinus nerve in two cases abolished the spontaneous fainting attacks

Studies on the Prevention of Cholesterol Atherosclerosis in Rabbits   By KENNETH B TURNER and GEORGE B KHAYAT (introduced by Randolph West), New York City, N Y
Atherosclerosis of the aorta was readily produced in rabbits by feeding cholesterol   The lesions were prevented in 17 of 19 animals by the simultaneous administration of dried whole thyroid gland with the cholesterol, while comparable thyroxin dosage was effective in only 3 of 10 rabbits   The changes produced by cholesterol were also prevented by potassium iodide in 17 of 18 animals   The bromide and chloride of potassium were ineffective   If complete thyroidectomies were performed upon the rabbits, potassium iodide no longer succeeded in preventing the intimal changes in the aorta   A close parallelism was observed between the level of the blood cholesterol and the presence or absence of arterial lesions

The Mitral Diastolic Murmur in Children without Mitral Valve Deformity but with Severe Rheumatic Carditis and Dilatation of the Left Ventricle By PAUL D WHITE and (by invitation) EDWARD F BLAND and T DUCKETT JONES, Boston, Mass

The Electrocardiogram in the Earlier Stages of Experimental Myocardial Infarction By FRANK N WILSON, PAUL S BARKER and (by invitation) FRANKLIN D JOHNSTON, IAN G W HILL, and GERALD C GROUT, Ann Arbor, Mich

A series of experiments was carried out in dogs in which various branches of the coronary arteries were ligated Electrocardiographic studies were made either immediately after the ligation, or at some later period within the first twenty-four hours In addition to the standard electrocardiographic leads and serial precordial leads, direct and semi direct leads from the exposed heart were employed

Immediately after ligation direct leads from the region supplied by the tied vessel yielded nearly monophasic responses, similar in all respects to those obtained from a region of the ventricular surface recently injured by burning or by other means The RS–T deviation shown by these monophasic curves rapidly diminishes but does not entirely disappear until the infarcted muscle dies some twelve or fifteen hours after ligation The curious T wave changes that occur in the coronary occlusion in man are also seen in direct leads from the margin of the infarcted area some hours after ligation They are apparently due to alterations in the recovery process in this region

Hypertension with Arteriolar and Glomerular Changes in the Albino Rat Following Subtotal Nephrectomy By J EDWIN WOOD JR, and (by invitation) CLAYTON ETHRIDGE, University, Va

Hypertension and renal insufficiency following partial nephrectomy in rats have been reported by Chanutin and Ferris It is the primary purpose of this study to describe the histological changes in the rat kidney subjected to functional strain by subtotal nephrectomy and to investigate a possible relationship between these changes and hypertension

One hundred and eighty rats have been fed on a balanced diet One hundred and fifty three of these have had 80 per cent of the kidney tissue removed and 27 control animals, simple laparotomy but no nephrectomy Direct carotid blood pressure readings have been made and autopsy performed at intervals varying from 1 to 267 days Of 153 animals with subtotal nephrectomy 68 have had blood pressures ranging from 70 to 139 mm Hg 42 from 140 to 169 mm Hg 32 from 170 to 199 mm Hg, and 11 from 200 to 230 mm Hg The higher blood pressure levels have been found in rats with subtotal nephrectomy of 60 days duration or longer and in each instance of hypertension degenerative glomerular changes have been present The blood pressure in 27 control rats varied between 100 and 140 mm Hg with an average of 120 mm. Hg, a figure already fully established by Chanutin and Ferris

During the early days of the experiment the tubules show rapid dilatation and epithelial hyperplasia with marked diffuse fatty change This tubular fatty change is transient but reappears in localized areas at a later stage Early, the glomeruli are distended and enlarged but free of degeneration.

Definite glomerular involvement begins to appear at approximately 40 to 70 days after operation as shown by marked epithelial cell enlargement with subsequent vacuolar degeneration and glomerular adhesions to the capsule. In

the older animals there is further glomerular degeneration with a gradual loss of structural detail and progression to varying degrees of hyalinization and fibrosis Glomerular fatty degeneration is striking and begins to appear in 80 to 120 days This is also progressive and is marked after 170 days Fatty degeneration of the afferent arterioles has been found at still later stages These time intervals may vary None of the pathologic changes has been seen in the control animals

Summary Hypertension frequently follows subtotal nephrectomy in rats Fatty degeneration in the glomeruli and afferent arterioles, glomerular atrophy and hyalinization and tubular dilatation following subtotal nephrectomy have been observed and may be interpreted as a natural sequence of hypertrophy and degeneration brought about by functional strain

The Response with Respect to Serum Volume and Composition after the Ingestion of Glucose in Diabetic Patients By F WILLIAM SUNDERMAN and (by invitation) ENNION WILLIAMS, Philadelphia, Pa

Blood was removed from diabetic patients, fasting, and one and one-half hours after the ingestion of glucose The increase in the glucose concentration in the serum per kilogram of water was found to be accompanied by a decrease in the chloride concentration in approximately the ratio of 3 8 mM to 1 If the assumption be made that the solids of the serum, excepting glucose and chloride, are constant and on this basis the amounts of glucose, water, and chloride per unit of the remaining solids are calculated before and after the administration of glucose, it is found that the increase in the glucose is accompanied by an increase in both water and chloride The solution transferred to the serum with the glucose is a hypotonic saline solution of an average chloride concentration of 60 mM per kilogram of water, but with the addition of the glucose, it has on the average a higher osmolar concentration than the serum The final result, therefore, is an increase in the glucose concentration in the serum, a decrease in the chloride concentration, an increase in the total osmotic pressure, and an increase in the serum volume This would indicate that in the serum of the diabetic patient the disturbance resulting from the uptake of glucose is distributed among at least three other variables —serum volume, osmotic pressure and chloride concentration

THE DIURNAL RHYTHM IN WATER AND MINERAL EXCHANGE [1]

By ROBERT C MANCHESTER

(From the Department of Pediatrics The University of Rochester School of Medicine and Dentistry Rochester New York)

In the course of investigations on total mineral and water exchange in epileptic children (1), a diurnal rhythm in the excretion of water and certain minerals was regularly encountered, which persisted even under stringently abnormal metabolic conditions   Recently, these observations have been confirmed and further elaborated in a series of studies on two mild epileptic patients and one normal male subject

It is common knowledge that urine is excreted in larger amounts during the day than at night, and that the increased urine volume of the day is associated with a negative water balance, counterbalanced at night by a positive balance due to a decrease in urine output   Associated with the period of retention, Simpson (2) found that an increase occurred in *urinary hydrogen ion concentration, titratable acidity and ammonia,* while chloride excretion fell below that of the day period

Norn (3) gave subjects equal amounts of food and water at three hour intervals throughout the day and night   The excretion of sodium, potassium and chloride was found to be lower at night than during the day, reaching a minimum between 3 00 A M and 6 00 A M and a maximum between 12 00 noon and 3·00 P M   The rhythm seemed to be dependent on the degree of activity   It was reversed when the subject slept through the day and worked through the night period

Other papers have appeared in the literature dealing with the excretion of various urinary constituents over day and night periods but as far as the author is aware a composite study is not available which includes gross water exchange and the more important urinary elements

PLAN OF STUDY AND METHODS

The greater part of the data to be presented has been selected from a series of investigations on two mildly epileptic male children carried out over a period of forty consecutive experimental days   No convulsions occurred throughout the entire study

Except for changes induced every effort was made to maintain the environmental and metabolic conditions as constant as possible not only from day to

day, but also for each period of the day   The subjects were kept in bed under sufficient bed covers to prevent chilling or sensible perspiration   Room temperature and relative humidity were recorded every two hours   Humidity ranged from 40 to 80 per cent and room temperature from 22° to 25° C , only occasionally rising high enough to induce sensible perspiration in the axilla   Activity was regulated and recorded by a trained nurse in constant attendance

Each day was divided into four periods of six hours beginning at 6 00 A M   At the beginning of each period, the subject voided, was weighed on scales sensitive to five grams and was then given an accurately prepared meal of known weight and composition

The four meals of the twenty-four hour period were identical in every respect and were prepared by a trained attendant in an adjoining kitchen from simple foods such as powdered milk,[2] 40 per cent cream, lactose, egg yolk, sodium chloride, and distilled water   A sufficient quantity of lactose and powdered milk was obtained to meet the needs of the entire study, thus reducing variations in the diet to a minimum   That metabolic and mineral requirements were adequately met is indicated by the uniformity in mineral and water excretion during control periods   The ketogenic antiketogenic ratio of the metabolic mixture was slightly below unity

Water balances were calculated by the method suggested by Newburgh, Johnston, and Falcon-Lesses (4)   The subjects were in nitrogen equilibrium and the caloric value of the food intake was approximately equivalent to the caloric requirement so that non-oxidation water freed by the catabolism of body tissue or stored with any protein or fat deposited in the body was disregarded   "Total water available" includes drinking water, preformed water of the foods, and water of oxidation of the metabolic mixture   "Total water loss" includes water of the urine, water of the feces, and water lost insensibly by way of the skin and respiratory tract

The water content of the urine and foods was determined by weighing 5 to 15 gram samples before and after desiccation in vacuo over sulphuric acid   The nitrogen of the foods, urine, and feces was determined by the Kjeldahl method (5)   Urinary chloride was determined by the Vohlhard method (5), inorganic phosphate by the method of Fiske and Subbarow (6), sulphate by the method of Fiske (7), titratable acidity by the method of Folin (8) and ammonia by the permutit method of Folin and Bell (9)   For the other mineral analyses urines were first ashed by treating with small amounts of nitric, perchloric, and sulphuric acids   Using the ashed specimens, sodium was determined by the method of Barber and Kolthoff (10), calcium by the gasometric method of Van Slyke and Sendroy (11), potassium by the method of Shohl and Bennett (12), and magnesium by a method adapted by the author which was based upon the precipitation of magnesium as magnesium ammonium phosphate and the measurement of the phosphate radicle by the Fiske and Subbarow method (6)   All samples of blood were drawn under oil without venous stasis

Unless otherwise stated, the subject followed a definite routine of activity consisting of games, reading and regulated naps from 6 00 A M to 6 00 P M , and slept from 6 00 P M to 6 00 A M except for interruptions resulting from the experimental routine   Since identical meals were taken at equal intervals throughout the day and night, all variables except activity and sleep and the resulting metabolic factors were reduced to a minimum

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[2] Klim

RESULTS OF EXPERIMENTS
Diurnal rhythm under standard metabolic conditions

The diurnal variations regularly encountered are clearly demonstrated in Table I   In Part I, a negative water balance is noted during the day period and a positive balance at night, dependent largely on the fluctuation in urine volume   Associated with the negative balance, urine volume, and urinary sodium, potassium, and chloride excretion reaches a maximum, while during the positive water balance of the night period, excretion falls to a minimum   A similar less significant rhythm occurs in titratable acidity, and phosphate, sulphate, and ammonia   "Total inorganic acid' and "total base" is greater during the day than at night   The difference is obviously largely dependent on the sodium, potassium and chloride fractions   Nitrogen excretion and body temperature are greater in the daytime than at night   Insensible perspiration shows no constant variation

In a more detailed experiment presented in Part II in which the day is divided into six hour periods, the same diurnal rhythm is demonstrated   The negative water balance, urine volume, and sodium, potassium, and chloride excretion reach a maximum in the morning period from 6 00 A M to 12 00 noon and begin to decline in the afternoon   Excretion decreases sharply through the night and reaches a minimum in the period from 12 00 midnight to 6 00 A M   coincident with the period of greatest water retention

In Table II, the data of Table I, Part I, have been recalculated to show the variation in concentration of the more important urinary constituents in day and night specimens   The molar concentration of those substances showing only a slight diurnal rhythm, namely phosphate, sulphate, and ammonia   increases slightly in the night urine   In the case of sodium, potassium, and chloride, the decrease in excretion during the night is parallel to a decrease in urine volume so that no regular change in the molar concentration of these elements occurs   Fluctuations in the total molar concentration of the inorganic ions are insignificant and the higher specific gravity of the night urine is due largely to an increase in the molar concentration of the nitrogenous substances, of which urea is the most important

The marked diurnal fluctuations in urinary sodium, potassium, and chloride excretion suggest the possibility of a corresponding change in the quantity of these elements in the blood   Unfortunately, only one experiment bearing on this point was carried out

The results, presented in Table III show no significant change in serum sodium or chloride in spite of a wide difference in urinary sodium and chloride excretion between the day and night period   Considered as a single experiment, no conclusions can be drawn   The results, however, are in accord with those of Norn (3)

TABLE I

The diurnal rhythm in water balance and the excretion of urinary constituents under standard conditions   H D, age 5 years   Weight 20 kgm

Time	Activity	Body temperature	Water balance				Composition of urine														
			Total water available	Total water loss	Balance	H₂O of insensible loss	Volume	Specific gravity	Nitrogen	Inorganic acid				Titratable acidity	Inorganic base					NH₃	Total inorganic base + NH₃ + titratable acidity
										Cl	PO₄	SO₄	Total		Na	K	Ca	Mg	Total		
			grams	grams	grams	grams	cc.		grams	cc n/10	cc n/10	cc n/10	cc n/10	cc n/10	cc n/10	cc n/10	cc n/10	cc n/10	cc n/10	cc n/10	cc n/10
PART I																					
6 A M – 6 P M	Active	36 8 37 7	751	930	−170	400	523	1 015	3 41	318	271	170	789	168	248	181	48	11	488	157	813
6 P M – 6 A M	Sleeping	37 5	730	617	+122	390	210	1 025	3 37	122	260	113	525	151	112	75	47	12	210	138	539
6 A M – 6 A M			1490	1547	−57	790	742		6 78	470	531	313	1311	322	360	256	95	23	714	295	1351
6 A M – 6 P M	Active	37 1 37 3	751	907	−156	279	621	1 010	3 43	315	255	173	743	152	206	180	42	8	430	158	710
6 P M – 6 A M	Sleeping	37 2	730	559	+140	317	253	1 021	2 96	137	247	137	521	145	120	78	48	12	258	157	560
6 A M – 6 A M			1490	1496	−6	596	871		6 39	453	502	310	1204	297	326	258	90	20	694	315	1300
PART II																					
6 A M – 12 N	Active	37 2	375	465	−90		301	1 015	1 69	152	135	82	369	80	81	119	21	5	226	76	382
12 N – 6 P M	Active	37 5	375	383	−8		223	1 013	1 6	101	140	80	327	83	64	91	22	5	182	70	335
6 P M – 12 M N	Sleeping	37 2	370	314	+56		160	1 010	1 58	80	126	77	283	72	37	48	25	6	118	65	254
12 M N – 6 A M	Sleeping	36 8	369	203	+70		163	1 010	1 54	64	133	70	278	81	25	47	25	5	102	65	248

TABLE II

Comparison of the molar concentration of urinary constituents in day and night periods Urea is calculated as 80 per cent of the urinary nitrogen

H D, age 5 years   Weight 20 kgm

Time	Volume	Specific gravity	Inorganic acid				Inorganic base					NH₃ concentration	Urea concentration	Total concentration
			Cl Concentration	P Concentration	S Concentration	Total Concentration	Na Concentration	K Concentration	Ca Concentration	Mg Concentration	Total Concentration			
	cc.		M per liter	M per liter	M per liter	M per liter	M per liter	M per liter	M per liter	M per liter	M per liter	M per liter	M per liter	
6 A M–6 P M	523	1 015	035	007	002	044	047	034	001	004	086	028	199	357
6 P M–6 A M	219	1 025	028	017	004	049	051	034	003	010	098	058	465	670
6 A M–6 P M	621	1 010	028	006	002	036	033	029	001	003	066	024	168	294
6 P M–6 A.M	253	1 021	027	014	003	044	047	031	002	010	090	058	356	548

TABLE III

Comparison of urinary sodium and chloride excretion with the level of sodium and chloride in the blood serum in day and night periods   R C M

age 25 years   Weight 63 kgm

Activity	Time	Water balance			Urine					Blood serum		
		Total water available	Total water lost	Balance	Volume	Specific gravity	Na	K	Cl	Time	Na	Cl
		grams	grams	grams	cc		mgm	mgm	mgm		mgm	mgm
Active	6 A M–6 P M	1548	1854	−306	1111	1 014	1500	2033	3442	12 noon	321	354
Sleeping	6 P M–6 A M	1548	1237	+311	723	1 022	1070	853	1476	12 midnight	326	350

The effect of activity on the diurnal rhythm was studied in a short experiment presented in Table IV   On the second day, the routine was altered so that the subject remained inactive, but awake, lying quietly on his back, from 6 00 A M to 12 00 noon   In spite of the reduced activity, the usual matutinal rise in urine volume, and sodium, potassium, and chloride excretion occurred   He was allowed to sleep as usual from 6 00 P M to 12 00 midnight but was kept awake and active from midnight to 6 00 A M   Coincident with the resumption of activity, water balance became negative, and urine volume, sodium, potassium, and chloride excretion increased   The subsequent sleeping period from 6 00 A M to 12 00 noon was unsatisfactory because of restlessness on the part of the subject   Urine and mineral excretion remained at about the same level as in the preceding period, but in spite of this they increased in the subsequent period in which normal activity was resumed

Although the experiment is not entirely satisfactory, the results are in accord with those of Norn (3) who was able to reverse the rhythm in subjects working at night and sleeping by day

The diurnal rhythm under abnormal metabolic conditions

A few experiments which were made under abnormal experimental conditions are presented in order to demonstrate the tenacity with which the rhythm is maintained even under extreme metabolic conditions

The data presented in Part I of Table V are selected from a period in which ingestion of water was being forced   In spite of the large urine output, the volume of urine of the day exceeds that of the night period and the diurnal fluctuation in sodium and chloride excretion persists

Even in a state of dehydration, as shown in Part II of Table V, the maximum water and mineral loss was found to occur in the day period   Urine volume, potassium, and chloride excretion reached a maximum during the day and the negative water balance of the day exceeded that of the night period   Sodium balance may be temporarily reversed, the excretion of the night period exceeding that of the day as it was found to be on the second day of the experiment

The results of a fasting period of two days duration are presented in Table VI   The usual rhythm in water balance, chloride, and sodium excretion was maintained and the small variations in phosphate and sulphate noted under normal conditions were more pronounced   Nocturnal excretion of potassium, ammonia, and titratable acidity was increased on the second day of fasting to the point at which it exceeded that of the day   Sodium, potassium, and chloride were stored during the recovery period   In spite of the demand of the body for the replenishment of depleted stores, the diurnal rhythm in excretion of these substances persisted

TABLE IV

The effect of changes in the routine of sleep and activity on the diurnal rhythm   N F age 10 years   Weight 27 kgm

Time	Activity	Water balance				Urine								Body temperature
		Total water available	Total water loss	Balance	Volume	Specific gravity	Nitrogen	Cl	P	S	Na	K		C
		grams	grams	grams	cc		grams	mgm	mgm	mgm	mgm	mgm		
6 A M – 6 P M	Active	833	1093	–260	843	1 009	4 19	1795	435	390	1024	712		
6 P M – 6 A M	Sleeping	832	736	+ 96	503	1 013	3 38	414	380	303	207	350		
6 A M –12 N	Flat on back Awake quiet	416	519	–103	426	1 013	2 19	1045	178	221	702	480		36 8
12 N – 6 P M	Active	416	506	– 90	329	1 012	1 98	570	168	240	316	390		37 2
6 P M –12 MN	Sleeping	416	360	+116	257	1 012	1 64	171	140	173	87	187		37 2
12 MN – 6 A M	Active	416	456	– 15	344	1 013	2 16	446	161	212	266	357		37
6 A M –12 N	Sleeping	416	433	– 17	310	1 012	2 25	400	185	260	228	339		37 5
12 N – 6 P M	Active	416	491	– 75	333	1 010	2 28	586	182	278	280	493		37 4
6 P M –12 MN	Sleeping	416	432	– 16	253	1 013	1 77	291	147	193	179	246		37 2
12 MN – 6 A M	Sleeping	416	380	+ 36	278	1 011	1 88	259	162	239	131	291		37 1

TABLE V

The influence of diuresis and dehydration on the diurnal rhythm   H D , age 5 years   Weight 20 kgm

Time	Activity	Body temperature		Water balance						Urine				
				Total water available	Total water loss	Balance	Volume	Specific gravity	Nitrogen	Na	K	Cl	NH₃	Titratable acidity
				grams	grams	grams	cc		grams	cc n/10	cc n/10	cc n/10	cc n/10	cc n/10

Part I Diuresis

Time	Activity	Body temperature		Total water available	Total water loss	Balance	Volume	Specific gravity	Nitrogen	Na	K	Cl	NH₃	Titratable acidity
6 A M–6 P M	Active	37 5	37 8	1900	1900		1520	1 006	3 68	139		274		173
6 P M–6 A M	Sleeping	37 4	37 2	1890	1823	+ 67	1140	1 004	3 07	97		110		118

Part II Dehydration

Time	Activity	Body temperature		Total water available	Total water loss	Balance	Volume	Specific gravity	Nitrogen	Na	K	Cl	NH₃	Titratable acidity
6 A M–6 P M	Active	37 1	37 3	751	907	− 156	621	1 010	3 43	206	180	315	158	152
6 P M–6 A M	Sleeping	37 2	37 2	738	589	+ 149	253	1 021	2 96	120	78	137	157	115
6 A M–6 P M	Active	37 4	37	208	561	− 353	212	1 030	3 55	198	196	295	134	157
6 P M–6 A M	Sleeping	37 6	37 2	195	405	− 210	163	1 035	3 33	238	111	236	106	160
6 A M–6 P M	Active	37 1	37 4	205	463	− 258	204	1 034	3 71	321	263	400	104	117
6 P M–6 A M	Sleeping	37 6	37 4	200	284	− 84	165	1 038	3 81	247	173	217	105	162

TABLE VI

The effect of fasting on the diurnal rhythm   N F   age 10 years   Weight 27 kgm

Time	Period	Water balance			Nitrogen	Specific gravity	Volume	Composition of urine													
		Total water available*	Total water lost	Balance				Inorganic acid				Beta oxy butyric	Titratable acidity	Inorganic base					NH₃	Inorganic base + titratable acidity + NH₃
								Cl	PO₄	SO₄	Total			Na	K	Ca	Mg	Total		
		grams	grams	grams	grams		cc	cc n/10	cc n/10	cc n/10	cc n/10	cc n/10	cc n/10	cc n/10	cc n/10	cc n/10	cc n/10	cc n/10	cc n/10	cc n/10
6 A.M–6 P.M	Fasting	906	1195	−289	3.63	1.010	962	344	252	202	798	2	120	282	222	40	8	552	111	783
6 P.M–6 A.M		905	723	+182	2.88	1.010	484	50	142	127	319	2	87	26	95	29	7	157	106	350
6 A.M–6 A.M		1811	1918	−107	6.51		1446	394	394	329	1117		207	308	317	69	15	709	217	1133
6 A.M–6 P.M	Fasting	914	1081	−167	3.81	1.009	852	101	268	200	569	146	213	186	233	53	7	479	137	829
6 P.M–6 A.M		913	874	+39	3.59	1.008	675	54	170	180	404	80	230	34	275	50	7	366	162	758
6 A.M–6 A.M		1827	1955	−128	7.40		1527	155	438	380	973	226	443	220	508	103	14	845	299	1587
6 A.M–6 P.M	Standard diet	838	1054	−216	4.87	1.011	703	197	281	229	707	5	186	111	163	65	10	349	210	745
6 P.M–6 A.M		837	692	+145	4.36	1.016	378	95	207	178	480	5	131	12	52	65	9	138	220	489
6 A.M–6 A.M		1675	1746	−71	9.23		1081	292	488	407	1187		317	123	215	130	19	487	430	1234
6 A.M–6 P.M	Standard diet	833	927	−94	4.10	1.010	723	254	235	204	693		141	136	112	67	11	326	207	674
6 P.M–6 A.M		832	630	+202	3.59	1.013	398	136	215	164	515		123	51	54	60	10	175	191	489
6 A.M–6 A.M		1665	1557	+108	7.69		1121	390	450	368	1208		264	187	166	127	21	501	398	1163
6 A.M–6 P.M	Standard diet	833	1108	−275	3.99	1.009	780	418	239	182	839		133	282	174	67	11	534	191	858
6 P.M–6 A.M		832	564	+268	3.33	1.014	418	100	189	166	455		104	39	63	67	10	169	163	436
6 A.M–6 A.M		1665	1672	−7	7.32		1198	518	428	348	1294		237	321	237	134	21	703	354	1294

* No allowance was made for water freed through the catabolism of body glycogen Preformed water freed by the catabolism of body protein and fat was calculated by the method suggested by Newburgh, Johnston, and Falcon Lesses (4)

The results of a study of a three day fast contained in Table VII show the same fluctuation in water, urine volume and sodium as in Table VI   Potassium balance was reversed on the second day of fasting, the excretion of the night period exceeding that of the day   The ability of the body to conserve base to replace depleted stores was strikingly

TABLE VII

The influence of a three-day fast and subsequent recovery period, on the diurnal rhythm
J R , age 15 years   Weight 40 kgm

Time	Period	Water balance				Sodium		Potassium	
		Total water available*	Total water lost	Balance	Urine volume	Intake	Urine	Intake	Urine
		grams	grams	grams	cc	mgm	mgm	mgm	mgm
6 A M –6 P M	Fasting	1140	1745	−605	1225		2795		942
6 P M –6 A M		1140	1030	+110	675		1305		503
6 A M –6 A M		2280	2775	−495	1900		4100		1445
6 A M –6 P M	Fasting	1050	1510	−460	942		1141		332
6 P M –6 A M		1050	1200	−150	800		790		823
6 A M –6 A M		2100	2710	−610	1742		1931		1155
6 A M –6 P M	Fasting	1040	1460	−420	807		890		1242
6 P M –6 A M		1020	1070	− 50	690		400		1066
6 A M –6 A M		2060	2530	−470	1497		1290		2308
6 A M –6 P M	Stand-	910	1020	−110	565	230	80	610	562
6 P M –6 A M	ard	910	1110	−200	490	230		610	94
6 A M –6 A M	diet	1820	2130	−310	1055	460	80	1220	656
6 A M –6 P M	Stand-	910	880	+ 30	365	230		610	143
6 P M –6 A M	ard	910	790	+120	405	230		610	61
6 A M –6 A M	diet	1820	1670	+150	770	460		1220	204

* Preformed water freed by the catabolism of body protein and fat was calculated by the method suggested by Newburgh, Johnston, and Falcon-Lesses (4)

demonstrated during the recovery period   Sodium almost disappeared from the urine   Potassium although diminished was excreted in larger quantities during the day than at night   Whatever the factors may be controlling the diurnal fluctuations, it is obvious that each element may be selectively influenced by other more fundamental physiological mechanisms, in this case, the demand of the organism for sodium to replenish depleted stores

DISCUSSION

The foregoing experiments furnish proof of a well established diurnal rhythm in water balance, urine volume, urinary sodium, potassium, and

chloride excretion, and specific gravity   The small fluctuations noted in urinary phosphate, sulphate, titratable acidity, and ammonia are of questionable significance, although there is almost uniformly a larger excretion of all these solutes during the day than at night   No significant change in calcium or magnesium occurs   The increased specific gravity of the night urine specimen is due largely to the nitrogenous fraction in spite of the fact that the total amount of urinary nitrogen excreted is slightly smaller during the night

As a rule the negative water balance, urine volume, and urinary sodium, potassium, and chloride output reach a maximum in the morning period from 6 00 A M to 12 00 noon, decline in the afternoon, and decrease sharply at night, water balance becoming positive, and volume of urine, and sodium, potassium and chloride excretion reaching a minimum in the period from 12 00 midnight to 6 00 A M, which is coincident with the period of greatest water retention   The results obtained are at variance with those of Norn (3) who found that the period of maximum excretion occurred from 12 00 noon to 3 00 P M   They are in accord with those of Simpson (2) who observed a large matutinal increase in urinary volume and chloride after waking

Under standard experimental conditions the rhythm is tenaciously maintained, although in abnormal conditions, such as dehydration and fasting, various constituents may be specifically influenced, for example sodium in dehydration and potassium in fasting

Throughout the entire study a rough parallelism has been observed between water balance and the sodium, potassium, and chloride which appear in the urine, the negative water balance of the day period coin ciding with the period of maximum urinary sodium, potassium, and chloride excretion, and the positive water balance of the night period coinciding with the period of minimum excretion   The largest negative water balance and sodium, potassium, and chloride excretion occur together from 6 00 A M to 12·00 noon and the largest positive water balance and smallest mineral excretion from 12 00 midnight to 6 00 A M This is of interest in view of the fundamental conception of Gamble, Ross, and Tisdall (13) who established the fact that a close quantitative relationship exists between mineral and water metabolism, a retention of water requiring a retention of minerals and vice versa   In the data recorded here only a qualitative rather than a quantitative relationship can be established   This is not surprising, however, in view of the technical difficulties encountered in accurately determining water balance over the short periods used   In general the results tend to uphold such a concept

Based on the fact that potassium is limited almost exclusively to intracellular fluid and sodium to extracellular fluid, Gamble, Ross, and Tisdall (13) have considered changes in potassium as indicative of changes

The results of a study of a three day fast contained in Table VII show the same fluctuation in water, urine volume and sodium as in Table VI. Potassium balance was reversed on the second day of fasting, the excretion of the night period exceeding that of the day. The ability of the kidney to conserve base to replace depleted stores was strikingly

TABLE VII

The influence of a three day fast and subsequent recovery period, on the diurnal rhythm. J. R., age 15 years. Weight 40 kgm.

Time	Period	Water balance			Urine volume	Sodium		Potassium	
		Total water available*	Total water lost	Balance		Intake	Urine	Intake	Urine
		grams	grams	grams	cc	mgm	mgm	mgm	mgm
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DISCUSSION

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                                        limited almost exclusively to
                                        lar fluid Gamble, Ross, and
                                        sium as indicative of changes

in intracellular water and changes in sodium as corresponding to those affecting extracellular water   Since sodium and potassium both take part in the diurnal rhythm, it may be assumed that both intra- and extracellular water and minerals enter into the changes noted

In general the fluctuations recorded seem to be dependent more on the difference in the state of consciousness, as between sleeping and waking, than upon variations in the degree of physical activity   Although it is dangerous to draw conclusions from a single experiment, the data presented in Table IV would tend to minimize the importance of the latter   On the second experimental day, in spite of the fact that the subject remained quiet and inactive flat on his back from 6 00 A M to 12 00 noon, the usual matutinal increases in urine volume, chloride, sodium, and to a lesser extent potassium, occurred, whereas with the resumption of normal activity in the subsequent period from 12 00 noon to 6 00 P M , excretion was materially diminished   The results are in accord with those of Norn (3) who found the rhythm was uninfluenced by wide differences in muscular activity

The mechanism involved remains obscure   Since the subjects were fed equal meals throughout the twenty-four hour period, differences in intake are excluded unless it be discovered that sleep interferes with intestinal absorption   That such is not the case is indicated by the fact that the rhythm persists in fasting

The variations in the metabolic rate between sleeping and the waking state could account for only minor fluctuations   In the experiments recorded, the metabolic rate, estimated from the metabolism tables of Atwater and Benedict, was usually approximately 15 to 20 per cent higher during the day from 6 00 A M to 6 00 P M than at night   Such an increase obviously provides for excretion of larger amounts of minerals and water of hydration and oxidation of catabolized substances during the day than at night   The difference in regard to water, however, amounts to only a few cubic centimeters and is insufficient to account for the large diurnal rhythm noted   Further evidence of independence of metabolic rate has been presented in Table IV in which the usual matutinal increases occurred in spite of the fact that the subject remained flat on his back and inactive, whereas in the subsequent period, when with the resumption of normal activity the metabolic rate increased, the values for urine volume and mineral excretion decreased

SUMMARY AND CONCLUSIONS

1 There is a diurnal rhythm in mineral and water balance characterized by the facts that urine volume and urinary sodium, potassium, and chloride excretion are greater during the day than at night   The larger urine volume of the day period is associated with a negative water balance, counterbalanced at night by a decreased urine volume and

positive water balance   Only slight fluctuations are noted in urinary phosphate, sulphate, titratable acidity, and ammonia, although there is almost uniformly a larger excretion of all these solutes during the day than at night   No significant shift in calcium and magnesium occurs "Total inorganic acid" and "total base" excretion are greater during the day than at night, due largely to the sodium, potassium, and chloride fractions

2   The negative water balance, urine volume, and urinary sodium, potassium, and chloride output reach a maximum in the morning period from 6·00 A M to 12 00 noon, decline in the afternoon, and fall off sharply at night, water balance becoming positive, and sodium, potassium, and chloride excretion reaching a minimum from 12·00 midnight to 6 00 A M , coincident with the period of greatest water retention

3   The rise in the specific gravity of the night urine is due largely to an increase in the molar concentration of the nitrogenous fraction, in spite of the fact that the total amount of urinary nitrogen excreted is slightly diminished during the night

4   A rough parallelism exists between water balance and urinary sodium, potassium, and chloride excretion   The negative water balance of the day period coincides with the period of maximum sodium, potassium, and chloride excretion and the positive water balance of the night period coincides with the period of minimum excretion   The largest negative water balance and sodium, potassium, and chloride excretion occur together from 6 00 A M to 12 00 noon and the largest positive balance and smallest excretion from 12 00 midnight to 6 00 A M

5   Since sodium and potassium both take part in the diurnal rhythm, it may be assumed that both intra and extracellular water and mineral metabolism contribute to the changes observed

6   Either urinary sodium or potassium excretion may be specifically influenced in abnormal metabolic conditions   In dehydration the diurnal rhythm in sodium is temporarily reversed with night excretion exceeding that of the day period, while in fasting potassium may be reversed

7   The mechanism involved in maintaining such a constant rhythm seems to be dependent upon the difference between the sleeping and waking states of consciousness rather than upon changes in the degree of physical activity, although the influence of the latter has not been adequately excluded

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THE EFFECT OF SPLENIC CONTRACTION ON THE FORMED ELEMENTS OF THE BLOOD IN A CASE OF ANEMIA AND SPLENOMEGALY

BY D. K. MILLER AND C. P. RHOADS

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The function of the spleen as a reservoir for the formed elements of the blood is a comparatively recent conception. Experimental studies have dealt principally with the number of erythrocytes stored or static in the splenic pulp and have neglected other equally significant cellular elements. Such studies moreover have been almost entirely made upon animals and the application of the results to problems of human physiology has not been proven.

Stukeley (1) suggested in 1723 that the spleen manifested relaxation and active muscular contraction. Gray (2) in 1854 mentioned the function of the spleen as that of a safety valve ' for the storage of blood apart from the general circulation. Roy was the first physiologist to observe the actual rhythmic movements and contraction of the spleen in laboratory animals (3). His work was confirmed in 1896 by Schafer and Moore who also demonstrated the innervation of the organ (4). The present day views of the function of the spleen as a blood reservoir are largely due to the work of Barcroft and his associates (5). Barcroft brought the spleens of dogs out through a slit in the abdominal wall and left them in this position for considerable periods of time protected only by saline dressings. With these preparations he demonstrated the rhythmic movements of the spleen and also the active contraction after various stimuli such as heat exercise and emotion. It was shown in later experiments that the spleen of the dog contracts to one half or one third of its normal size during exercise and to an even greater extent after severe hemorrhage or the death of the animal. His work with carbon monoxide has clearly demonstrated that the spleen serves as a reservoir of red blood corpuscles.

Many studies have been made on the hemoglobin content and the erythrocyte count of blood expelled from the spleen both during active contraction and while the organ was quiescent. Consistent results have not been obtained. In some cases identical values were obtained for blood from the splenic artery and vein. Cruickshank (6) working in Barcroft's laboratory by cannulizing the inferior mesenteric vein col

lected blood during contractions of the spleen   At the same time he measured the contraction of the spleen and the quantity of blood expelled From experiments of this nature he determined that the blood expelled was often richer in hemoglobin than was the blood of the general circulation   The concentration of hemoglobin varied during the contraction, the maximum value being 20 to 40 per cent higher than that of the normal peripheral blood   The amount of blood expelled from the spleen by a single contraction was estimated to be 2 6 to 5 6 per cent of the total blood volume of the animal

An opportunity for the detailed study of the spleen as a reservoir of blood was presented to us in a patient in which there was an anemia and splenomegaly   The spleen in this patient was easily demonstrable and could be visualized by the x-ray   It was observed that the spleen invariably contracted following the intravenous injection of liver extract, a therapeutic measure instituted with a view to relieving the anemia The present paper deals with the changes in the number and character of the formed blood elements which took place during the splenic contractions induced by this and other methods

METHODS

The general plan was to study the character and cellular composition of the peripheral blood before and after the induced contraction of the spleen, and to compare these findings with the changes in the size of the organ, as determined by x-ray

Blood samples were taken by venipuncture both before and after the splenic contraction   The red and white corpuscles were enumerated in the usual way in these samples   Hemoglobin determinations were made by the Sahli acid hematin method, using calibrated tubes and standards   The mean corpuscular volume of the erythrocytes was determined with the Wintrobe hematocrit tube   Enumeration of the blood platelets was then done on blood obtained from a freely bleeding puncture of the ear lobe   Three per cent sodium citrate solution prepared freshly every day was used as a diluting fluid in the red corpuscle pipette   Great care was exercised to keep the sodium citrate solution and all the glassware free from dust and dirt   Fragility tests of the red corpuscles were done by the usual method, using sodium chloride solutions in dilutions varying from 0 52 to 0 28 per cent   Controls were made on normal bloods at each determination   Blood volume determinations by the vital red method of Rowntree (7) were made before and after splenic contractions   X-rays of the spleen were taken before the injection and at regular intervals following the contractions   The surface area of the splenic shadow was measured on these x-rays with a Keuffel and Esser planimeter   The upper pole of the spleen could not be definitely outlined   therefore identical points were taken on the ribs from which measurements were made

OBSERVATIONS

Clinical summary   The patient a 55 year old Puerto Rican woman entered the hospital complaining of attacks of diarrhea and abdominal discomfort of four and a half years duration   A diagnosis of sprue had been

made fifteen years ago while the patient was a resident of Puerto Rico   Following her arrival in this country thirteen years ago the diarrhea ceased and the patient gained weight   There was no history of yellow fever, typhoid or malaria

The present illness began about four and a half years ago when the patient noticed five or six loose bowel movements a day   These attacks lasted about three weeks and occurred once or twice a year   An abdominal mass was noticed on the left side of her abdomen three years ago   Six months before entry the patient noticed shortness of breath and pallor   Three months later a local doctor found a severe anemia and transfused the patient   Following this the patient improved and was well until one month before entry when her diarrhea and abdominal discomfort returned   At the same time her pallor was again noticed and she became short of breath on exertion

Physical examination   The patient was markedly emaciated and had moderate pallor of her skin and mucous membranes   No lymphadenopathy was present   The heart and lungs were normal   The abdomen was moderately distended   A large hard mass was palpated on the left side of the abdomen   The lower edge of the mass extended to the level of the umbilicus the medial edge extended to the mid abdominal line   A notch was felt in this medial border   The red blood count was 3 240 000 hemoglobin 71 per cent white cell count 1 250   The differential counts done with Wright's stained smears and by the supravital technique showed no abnormal cells   The fragility of the red blood cells was within normal limits   Gastric analysis showed no free hydrochloric acid after histamine expression

For the treatment of the anemia liver extract was given intravenously During the first injection which was of twenty minutes duration the patient complained of severe abdominal pain localized over the splenic area   Nausea and vomiting promptly occurred   Toward the end of the injection marked flushing of the face neck hands and arms was present   Immediately following the injection palpation of the abdomen revealed that the spleen the lower edge of which had been just below the level of the umbilicus had contracted in size until its lower edge was palpable about 1 0 cm below the costal margin Moreover whereas the spleen had been hard before injection it was found to be very soft in consistency after the shrinkage in size   This observation suggested the studies reported in this communication   Table I represents a typical chronological protocol of these studies

The results obtained in a typical study using liver extract by intra venous injection as a stimulant to splenic contraction are shown in Table II   From this table it is evident that without essential change in the total blood volume there was a significant increase in the formed elements of the blood following a shrinkage in the size of the spleen Twenty four hours later the spleen had practically regained its original size   Figure 1 presents tracings of the splenic shadow taken from the x rays and demonstrates the change of size of the spleen   The area of the spleen before injection was 90 3 sq cm   Immediately after injection the spleen had contracted to an area of 32 3 sq cm   Accompanying this contraction there was an increase of 520 000 red blood cells in the erythrocyte count   The hemoglobin increased 15 per cent   At the same time even more marked rises were found in the white count and

TABLE I

Chronological protocol

Time	Remarks
2 45	Palpation of abdomen reveals a firm hard spleen, the lower edge of which is at the level of the umbilicus
2 58	X-ray of spleen
3 00	First bleeding Blood pressure 120/70
3 03	Platelet count
3 05	11 0 cc 1 5 per cent vital red solution given intravenously
3 09	Bleeding for blood volume
3 10	Injection of 10 0 cc liver extract Eli Lilly (or 20 cc Parke Davis) begun Blood pressure 122/68
3 15	Patient complained of severe pain in abdomen, headache and nausea No vomiting
3 17	Marked flushing of face has occurred
3 20	Blood pressure 84/44
3 25	Injection completed Spleen palpable about 2 0 cm below the costal margin
3 26	X-ray of spleen
3 28	Bleeding
3 30	Platelet count
3 38	11 0 cc 1 5 per cent vital red solution given intravenously
3 42	Bleeding for blood volume
3 45	X ray of spleen
24 hours later	x ray of spleen

the platelet count, the increase of the former being 950 cells and of the latter 74,000   Determination of the blood volume after contraction of the spleen showed an increase of the total blood volume of only 200 cc With this the plasma volume decreased from 65 to 60 per cent   There was also a decrease in the total plasma volume   The fragility of the red cells to sodium chloride solutions was not altered after contraction of the spleen

TABLE II

Effects of contraction of the spleen induced by liver extract

	Area of spleen	Red count	Hemo globin	White count	Mean corpus cular volume	Color index	Platelet count	Blood pressure
	sq cm	millions	per cent		μ³			
Patient								
Before injection	90 3	3 72	70	1400	0 870	0 950	49 000	120/70
After injection	32 3	4 24	85	2350	0 865	1 00	123,000	95/60
24 hours later	89 1	3 67	69	1150	0 860	945	52 000	
Control								
Before injection		2 68	67	4850	1 26	1 25	152,000	115/65
After injection		2 57	66	4500	1 28	1 28	138,000	90/60

	Total blood volume	Total plasma volume	Plasma per cent
	cc	cc	
Before injection	3,800	2 520	65
After injection	4,000	2,410	60
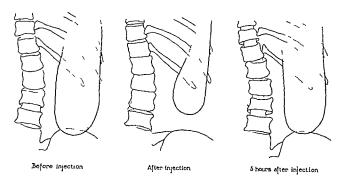
FIG 1

Shows a series of tracings taken from the x rays of the spleen demonstrating the size of the spleen immediately before the injection of liver extract immediately after contraction had occurred and 5 hours later at which time the spleen had practically regained its original size

A study of the formed elements of the blood following the injection of liver extract intravenously into a control patient with anemia, but without splenomegaly is also included in Table II   It is evident from these results that there was no appreciable alteration in these elements following this injection   Results similar to those found in Table II have been obtained repeatedly following the intravenous injection of liver extract into the patient with splenomegaly   The degree of con traction of the spleen and the changes in the formed elements of the blood have varied directly with the degree of contraction obtained in each study   The observations presented here are neither the maximal nor the minimal results obtained but are taken as the most typical

The combined results of the alterations of the formed elements in the peripheral blood obtained after induced splenic contraction in five studies in which liver extract was used as the stimulant are presented in Table III   The average splenic contraction as shown was from a sur face area of 93 6 sq cm to an area of 50 0 sq cm   This is a decrease in the size of the spleen to 53 per cent of its original size   The average increase in the erythrocyte count after contraction was 500 000 red cells or an increase of 13 9 per cent   Together with this the hemoglobin increase in this series was 10 per cent, or an average increase of 13 7 per cent of the original average hemoglobin value   The white blood cells showed an average increase of 840 cells or average increase of 51 per cent   The blood platelets showed an average increase of 66 000 or of 105 per cent   It is apparent from this table that the relative increases were far greater for the white cells and the blood platelets than for the erythrocytes this observation was seen in every study

FIG 2

This is an x-ray of the spleen taken immediately before the injection of liver extract   In this x-ray the lower border of the spleen is seen beneath the crest of the ilium

TABLE III

Combined studies of splenic contractions induced by liver extract

	Area of spleen	Red count	Hemo globin	White count	Platelet count
	sq cm	millions	per cent		
Before injection	93 6	3 62	73	1660	63 000
After contraction	50 0	4 12	83	2500	129 000
Per cent increase		13 9	13 7	51 0	105 0

These observations suggested the possibility of attempting to evaluate on the human spleen the effects of certain drugs which have been known

After injection

FIG 3

This is an x ray of the spleen taken immediately after the injection of liver extract and demonstrates the change in size of the splenic shadow

to stimulate splenic contractions in animals Consequently adrenalin, histamine and eserine were used to induce splenic contractions The results obtained following the intramuscular injection of these drugs are presented in Table IV

As shown in the table a marked contraction of the spleen was noted eight minutes after the intramuscular injection of 1 0 cc of adrenalin The spleen contracted from an area of 109 0 sq cm to an area of 48 7 sq cm One hour later it had increased in size to 82 4 sq cm Accom panying this contraction there was an increase in the red blood cell count of 650 000 cells together with an increase of hemoglobin of 19 per cent In this study the white blood cells showed the maximal increase obtained

TABLE IV

Effects of contraction of the spleen induced by adrenalin, histamine and eserine

	Area of spleen	Red count	Hemo globin	White count	Mean corpuscular volume	Color index	Platelet count	Blood pressure
	sq cm	millions	per cent		μ³			
Adrenalin								
Before injection	109 1	3 22	63	1500	0 917	0 985	90,000	121 75
8 minutes after injection of 1 cc , I M *	48 7	3 87	79	4100	0 925	1 01	158 000	138 80
1 hour after injection	84 2							
Histamine								
Before injection	100 8	3 32	65	1650	0 915	0 985	68 000	122,78
7 minutes after injection of 1 mgm I M	75 5	3 67	70	2700	0 915	0 960	124 000	106/52
1 hour after injection	93 5							
Eserine								
Before injection	97 5	3 37	64	1250	0 900	0 955	96 000	128 70
40 minutes after injection of 2 4 mgm I M	73 6	3 36	64	1100	0 890	0 955	88 000	115 75
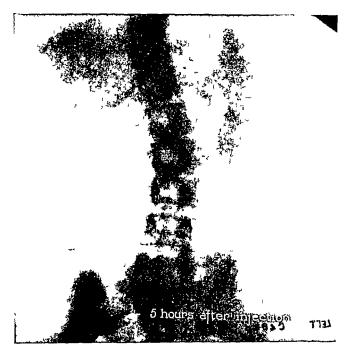
* I M —intramuscularly

namely, 2,600 cells, or an increase of 172 per cent With this contraction there was also an increase in the platelet count of 68,000

Splenic contraction was also induced by the intramuscular injection of 1 0 mgm of histamine Seven minutes after this injection the spleen had contracted from 100 8 sq cm to 75 5 sq cm In one hour it had increased in size to 93 5 sq cm , that is, almost to its original size With this contraction there was an increase in the red cell count of 350,000 red cells, with a 5 per cent increase of hemoglobin The white blood cells increased by 1,050 cells and the platelet count was increased by 56,000 platelets As in the previous studies the white blood cells and platelets showed a greater proportional increase than the red cells and the hemoglobin

The intramuscular injection of 2 4 mgm eserine sulphate also induced a splenic contraction This contraction did not occur until 40 minutes after the injection The surface area of the spleen decreased from 97 5 sq cm to 73 6 sq cm As seen in Table IV there was no appreciable alteration in the formed elements of the blood accompanying this contraction This was the only study in which no significant alteration of the formed elements of the blood was obtained with an induced splenic contraction When the parasympathetic nervous system was blocked by giving atropine intramuscularly no contraction followed the injection of eserine

Differential blood counts are found in Table V There was a relative and absolute increase in the polymorphonuclear cell count after the

FIG. 4

This x ray of the spleen was taken 5 hours after the contraction had occurred. It demonstrates the return of the spleen to its original size.

TABLE V

Differential white counts

	Contraction induced by liver extract		Contraction induced by adrenalin	
	Before	After	Before	After
	per cent	*per cent*	*per cent*	*per cent*
Polymorphonuclears	46	72	44	57
Eosinophils				1
Small lymphocytes	51	22	52	37
Large lymphocytes	2	2	2	2
Monocytes	1	4	2	3

induced splenic contraction   Accompanying this there was a relative
decrease in the lymphocyte count   These variations were found with
each splenic contraction studied   No abnormal cells were seen in the
smears taken either before or after the contraction   The reticulocyte
count showed no variation

The blood pressure was followed in each study, as may be seen in
Tables II and IV   A fall in blood pressure was invariably obtained fol-
lowing the intravenous injection of liver extract   A similar fall of blood
pressure occurred after the injection of histamine   In the studies in
which adrenalin and eserine were used as the stimulant to contraction,
there was invariably a rise in blood pressure

DISCUSSION

Before discussing the data presented in this paper it may be well to
point out that while certain changes in blood cytology took place as a
result of the induced splenic contractions, the results may not be indica-
tive of the function of the normal spleen, since it may be recalled that
the patient showed both an anemia and a splenomegaly   Obviously
such an abnormal organ could store a much greater quantity of blood or
cells than could a spleen of normal size   Furthermore, the degree or
ratio of contraction obtained could scarcely be expected to occur with
the normal spleen   Thus it might be thought that the pathological
conditions might negate or partially invalidate the significance of these
observations as regards the normal physiological function of the organ
as a blood reservoir   This matter cannot be definitely settled at this
time, but the authors prefer the interpretation that the present circum-
stances constitute merely an exaggeration of the normal

The mechanism of the contractions is not clearly understood   Dale
and his co-workers (8) isolated histamine from alcoholic extracts of fresh
liver   Histamine present in the liver extract given intravenously might
then be the substance responsible for the induction of the splenic con-
traction   Similar effects, namely a splenic constriction, were produced
with liver extract and with a drug stimulating the sympathetic nervous
system, adrenalin   In these two types of experiments, there was a
lowering of the blood pressure with the liver extract and a rise in blood
pressure accompanying the contraction induced by adrenalin   This
would seem to eliminate the fall in blood pressure as a significant occur-
rence   Furthermore, whereas there occurred a peripheral vasodilation
in the study with liver extract, and a peripheral vasoconstriction in the
contraction induced with adrenalin, it may be safely stated that peripheral
vasodilation had little or no effect on the production of the alteration in
the blood elements

The contraction induced by histamine may have been due to the
direct action of the drug on the unstriated musculature of the organ   or
it may have been the response of the spleen to shock produced by the drug

A drug which stimulates the parasympathetic nervous system namely, eserine, also gave a splenic contraction It may be pointed out that the contraction was smaller than that obtained by other means, and that there was no alteration in the blood elements accompanying the change in size of the spleen

Blood volume determinations showed very little alteration There may have been at other times a greater increase in blood volume not detected by the method used Vital red cannot be used repeatedly intravenously in clinical studies

Perhaps the most striking fact among these results is the disproportionate increase in cell counts Thus in each of the studies with liver extract and also with the other stimulating drugs the increase in the number of white blood cells was over threefold that of the red blood corpuscles, while the proportionate increase of the blood platelets was almost invariably greater than that of the white blood cells No matter what mechanism was involved in the addition of these cells to the peripheral blood, these ratios may represent the relative proportions of these cells available in the splenic reservoir This being the case the spleen may be considered as a potential and readily available source not only of red blood corpuscles but of white blood cells and blood platelets as well It is not inconceivable that the spleen functions as a reservoir for these cellular elements

SUMMARY

1 In a patient who showed both anemia and a splenomegaly it was possible to induce [and observe] a marked contraction of the spleen by the intravenous injection of liver extract and the intramuscular injection of adrenalin histamine and eserine This contraction was observed

2 Accompanying these induced splenic contractions there occurred marked increases in the number of cellular elements of the circulating blood The relative proportion of the various cellular components suggests that the blood of the splenic reservoir has a cytological composition in which the relative proportion of cells is different from that of the circulating blood

3 These experiments make it obvious that the spleen must be considered not only as a reservoir of red blood cells, but that it can also store proportionally greater numbers of white blood cells and blood platelets

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THE PROTEINS OF BLOOD AND SUBCUTANEOUS LYMPH IN DOGS

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In the course of experiments undertaken to discover the effect of the flow and the composition of lymph on the formation of edema an opportunity has been afforded for comparing the albumin and globulin concentrations of blood serum with those of lymph serum obtained from lymphatics of the extremities of dogs The comparison forms the basis of this communication

METHODS

The dogs used were of mongrel breeds and varied in weight from 15 to 26 kilograms Lymph was obtained through cannulas inserted into one of the lymphatic trunks just above the ankle of either a front or a hind leg Cannulization was performed sometimes under ether anesthesia, sometimes under nembutal anesthesia and sometimes with the aid of a local anesthetic (novocaine) only With animals under general anesthesia the flow of lymph was promoted by gentle massage of the foot and ankle and passive motion of the extremity The non anesthetized dogs were prepared by the method described by White Field and Drinker (1) and allowed to walk about the corridors the normal motion of the extremity furnishing the stimulus for lymph flow A fine fibrin clot which formed in the lymph on standing was removed before analysis Blood was obtained by puncture of the femoral artery usually at the end of the period of lymph collection and the serum separated as soon as possible In several instances blood serum obtained both before and after the collection of lymph failed to indicate any change in composition during the experiment

Methods of chemical analysis have been described in a previous paper (2) In some instances, because the quantities of lymph were limited nonprotein nitrogen was determined on the serum only and the value so obtained used in calculating the lymph proteins The error involved in such an assumption is small In nine instances in which parallel determinations were made of lymph and serum nonprotein nitrogen the average value for lymph was 24 5 mgm per 100 cc and for serum 22 7 mgm per 100 cc

ANALYTICAL RESULTS

The results of 25 parallel estimations of lymph and serum albumin and globulin made on specimens obtained from 13 dogs are shown in Table 1 A comparison of the albumin globulin ratios is shown graphically in Chart 1   Table 1 shows that the A/G ratios for serum varied from 0 5 to 2 0   The results may be regarded as accurate at least to the first decimal as the relatively high protein content of serum means that small analytical errors are not reflected in significant variations in the A/G ratio   On the other hand the A/G ratios for lymph varied between 0 1 and 3 0   In this case several specimens of lymph were encountered

TABLE 1

Proteins of blood serum and lymph from the legs of dogs

Data grouped by experiments and arranged roughly in ascending order of the total protein content of lymph

Dog num ber	Albumin		Globulin		Total protein		$\frac{Albumin}{Globulin}$		Remarks*
	Serum	Lymph	Serum	Lymph	Serum	Lymph	Serum	Lymph	
	grams per 100 cc	grams per 100 cc	grams per 100 cc	grams per 100 cc	grams per 100 cc	grams per 100 cc			
5	1 36	0 05	1 88	0 17	3 24	0 22	0 72	0 29	Loc—Rl—Nutritional edema
839	2 36	0 03	3 18	0 29	5 54	0 32	0 74	0 10	Nem—Rl
6	1 40	0 07	1 40	0 08	2 80	0 15	1 00	0 99	Nem—La—Plasmapheresis edema
	1 40	0 24	1 40	0 08	2 80	0 32	1 00	3 00	Ra—Gentle massage
	1 40	0 28	1 40	0 41	2 80	0 69	1 00	0 69	Ra—Vigorous massage
895	3 57	0 30	1 83	0 15	5 40	0 45	1 95	2 00	Nem—Rl
	3 57	0 48	1 83	0 27	5 40	0 74	1 95	1 78	La
1000	2 34	0 21	3 36	0 57	5 70	0 78	0 70	0 37	E—3 hours after ether Dog walking
949	2 99	0 53	3 05	0 50	6 04	1 03	0 98	1 06	Nem—La—Veins constricted
	2 99	0 44	3 05	0 29	6 04	0 73	0 98	1 52	La—Veins not constricted
58	3 44	0 49	2 26	0 30	5 70	0 79	1 52	1 63	E—Ra—Mas age while recovering
	3 44	0 64	2 26	0 37	5 70	1 01	1 52	1 73	Ra—Dog walking
	3 44	0 60	2 26	0 30	5 70	0 90	1 52	2 00	Ra—Dog running
	3 44	0 60	2 26	0 34	5 70	0 94	1 52	1 77	Ra—Walking after rest period

TABLE 1 (continued)

Dog number	Albumin		Globulin		Total protein		Albumin/Globulin		Remarks*
	Serum	Lymph	Serum	Lymph	Serum	Lymph	Serum	Lymph	
	grams per 100 cc	grams per 100 cc	grams per 100 cc	grams per 100 cc	grams per 100 cc	grams per 100 cc			
69	3 50	0 68	2 27	0 58	5 77	1 26	1 54	1 17	E—After recovery dog walking
840	3 08	0 97	1 99	0 52	5 07	1 49	1 55	1 86	Loc—Rl—Massage
	3 08	0 96	1 99	0 45	5 07	1 41	1 55	2 13	Rl—Walking
	3 08	1 16	1 99	0 49	5 07	1 65	1 55	2 37	Rl—Running
	3 08	0 86	1 99	0 41	5 07	1 27	1 55	2 10	Rl—Walking after rest period
91	3 75	1 15	2 63	0 74	6 38	1 89	1 43	1 55	Loc—La—Mixture walking and running
841	3 82	0 87	3 01	0 68	6 83	1 55	1 27	1 28	Nem—Ra
	3 82	1 14	3 01	0 91	6 83	2 05	1 27	1 25	La
	3 82	1 35	3 01	0 82	6 83	2 17	1 27	1 22	Rl and Ll
842	3 54	1 73	3 86	1 50	7 40	3 23	0 92	1 15	Nem—La—Veins constricted
950	2 49	0 85	5 40	2 60	7 89	3 45	0 46	0 33	Nem—La

* Abbreviations as follows Nem = nembutal anesthesia E = ether anesthesia Loc = local novocaine anesthesia La = left fore leg Ra = right fore leg Ll = left hind leg Rl = right hind leg

in which the total protein concentration was less than 0 5 gram per cent and it is evident that small analytical errors in the analysis of such specimens produce wide variations in the A/G ratios Moreover the ratios will increase in accuracy as the protein content of the specimen rises[1] This factor of the accuracy of measurement has been given consideration in Chart 1 Collectively considered there were 10 instances in which the serum ratio was greater than the lymph ratio and 15 examples of the reverse relationship The average albumin globulin ratio of all the lymph specimens analyzed was 1 41 ± 0 09   and the

[1] An example will render the magnitude of the possible analytical error more clear Duplicate analyses of a lymph specimen show for total protein 0 38 and 0 42 gram per cent and for albumin 0 18 and 0 22 gram per cent The A/G ratio calculated from the average of the duplicate measurements is 1 00 and yet the individual measurements can be combined to give ratios which vary from 0 75 to 1 38 An analytical variation of the same magnitude occurring in serum with a protein content of 6 00 grams per cent produces a ratio fluctuation between 0 98 and 1 02 only
    Probable error of the mean
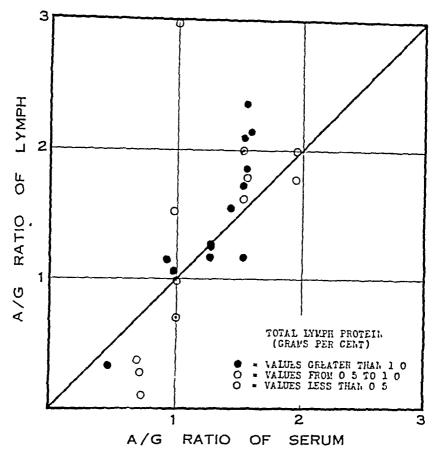
FIG 1   RELATIONSHIP BETWEEN THE ALBUMIN   GLOBULIN RATIOS OF BLOOD SERUM AND LYMPH FROM THE EXTREMITIES IN DOGS
The diagonal line follows the course of identity in the ratios and serves to emphasize the divergence of individual determinations from this identity

corresponding average for the serums was $1\ 26 \pm 0\ 05$° A statistical calculation by the product-moment formula gives for the respective ratios a coefficient of correlation of $0\ 81$ with a probable error of $0\ 05$ The correlation is sufficiently high to demonstrate a definite relationship between the albumin   globulin ratios of lymph and serum

DISCUSSION

An examination of pertinent literature, tabulated exhaustively by Drinker and Field (3), discloses a great number of observations of the total protein content of lymph while at the same time revealing a paucity of information concerning the protein fractions which enter into its composition   In 1891 Munk and Rosenstein (4) reported two analyses of lymph obtained from the leg of an 18-year-old girl who was afflicted with progressive elephantiasis and who presented a permanent lymph fistula over the upper portion of her calf   Both analyses revealed a total

protein concentration of 3 5 grams per cent, in one the albumin globulin ratio was 4 and in the other 2 4 Simultaneous analyses of the serum proteins were not made In 1906 Morawitz (5), in attempting to obtain information concerning the origin of the plasma proteins, made two experiments on dogs in which the proteins of plasma were compared with those of thoracic duct lymph In the first experiment the blood serum showed an albumin globulin ratio of 1 25 and the lymph serum a ratio of 1 56, in the second experiment the ratio for blood plasma was 1 44 and for lymph plasma 1 58 Although in both instances the lymph ratios were slightly higher than the blood ratios, the author did not regard the differences as significant Finally in 1932 Wells (6) reported nine experiments in which the albumin globulin ratios of serum were compared with those of lymph obtained from the mesenteric lacteals of dogs The measurements revealed an average albumin globulin ratio for serum of 1 18, an average ratio for lymph of 1 39, and agreed satisfactorily with direct estimations of the colloidal osmotic pressure The analyses reported here are the first in which the protein fractions of serum have been compared with those of lymph from vessels which drain the subcutaneous tissues Measurements of the colloidal osmotic pressure of serum and cervical lymph have, however, been reported by Loëwen, Field and Drinker (7) The osmotic pressures per gram of lymph protein varied from 55 to 103 mm of water and in each instance were considerably higher than the corresponding pressures for serum protein The excess pressure of lymph was thought to be an expression of greater permeability of the blood capillaries for serum albumin An approximate calculation made by us on the basis of Govaerts' factors (8) indicates that several of the recorded pressures for cervical lymph are so high that they could not be accounted for even if all of the lymph protein were in the form of albumin To explain them one would have to suppose either that lymph protein did not arise from serum protein or that the blood capillaries exhibit selective permeability for the smaller molecules of the albumin complex A selective permeability of this nature is not in agreement with the analyses reported here

The data reported in this paper show definitely that the albumin globulin ratios of serum and lymph are mutually dependent variables and are thus in accord with the view of Drinker and Field (3, 9) that lymph protein is derived from blood protein The correlation between the two variables is sufficiently high to indicate that the serum ratio is at least a major factor in establishing the lymph ratio It does not exclude the possibility that minor factors, among them a slight degree of selective permeability on the part of the blood capillaries, may prevent identity in the proportions of proteins in the two fluids Several of the grouped analyses obtained in single experiments in this study (particularly with dogs 58 and 840) strongly suggest the modifying influence of

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inquiry concerning the distribution of the capillary filtrate   We have already suggested that a large proportion of capillary tufts may be impermeable to protein   These tufts will yield a protein-free ultra-filtrate, the volume of which will depend upon the balance of pressures operating across their walls, namely, the colloidal osmotic pressure in serum together with capillary blood pressure on the inside of each tuft and mechanical tension in the intercellular spaces on the outside   The latter tension may be suspected of varying under conditions which affect the mobility of fluid in adjacent lymphatics, being decreased when the pumping action of the lymphatic valves is stimulated by exercise or massage and increased when the valves are at rest   The factors controlling the volume of a filtrate are, therefore, multiple and it is easy to conceive of circumstances, some pathologic and others purely physiologic, under which it will increase greatly or practically vanish   For present purposes it is important to hold in mind that this group of capillary tufts will be surrounded by an interstitial fluid which is essen-tially protein free and that some of this fluid, at times much and at times little, will find its way into radicles of the lymphatic system   Across the walls of other capillaries, those for example which permit the passage of protein, the course of events will be considerably modified   These vessels will yield interstitial fluid rich in protein which will act to diminish the effective osmotic pressure of the intracapillary colloids and therefore to increase the volume of filtrate   This filtrate will likewise find its way into adjacent radicles of the lymphatic system   To what extent diffusion through neighboring intercellular spaces will occur is a matter of pure conjecture but it may be supposed that so long as the lymphatics offer the path of least resistance the major portion of filtrate will move by this route   Among a large group of lymphatic capillaries we may therefore imagine that many are carrying a centripetal stream of lymph of ex-tremely low protein content and that a few are transporting fluid the protein concentration of which approaches that of the serum   The rate of lymph flow from the various radicles will, moreover, be more rapid in those which contain fluid of high protein concentration   Lymph, however, collected from any of the larger lymphatic trunks will represent a mixture of the streams coming from different radicles   Its composition may be expected to vary considerably as regards total protein content but the proportions of the several protein fractions should remain roughly the same as those in the serum of the same animal   An exceptional blood capillary which exhibited selective permeability for albumin molecules could produce a minute flow of this protein fraction into the general stream and so raise the A/G ratio slightly above that in the blood   The theory then is consistent with the experimental observations being re-ported   It cannot, however, be reconciled with the readings of colloidal osmotic pressure of Loewen, Field, and Drinker (7) which indicate marked divergence between the A/G ratios of serum and lymph

It will have been observed that the first theory, as outlined previously, can be identified in essential respects with the second if one supposes each individual blood capillary loop to possess pores the sizes of which cover the whole range of permeability which is assumed in the second theory for many capillaries The distinction, however, is of fundamental importance for in the former instance one must imagine an interstitial fluid everywhere identical in composition with lymph and in the latter it can be thought that one very important function of the lymphatic system is to prevent interstitial fluid from attaining the relatively high protein concentrations of lymph It is for this reason, chiefly, that the two theories have been outlined Although either is consistent with the results reported, it seems desirable to insist that the available experimental evidence does not yet justify the conclusion that lymph as it is collected from a large lymphatic trunk is identical in chemical composition with interstitial fluid in the major portion of the region drained by the trunk

SUMMARY

1 Experiments are reported in which the albumin and globulin concentrations in the serum of the blood are compared with those in lymph obtained from lymphatics of the legs of dogs

2 In 25 parallel estimations of lymph and serum albumin and globulin the respective A/G ratios showed a correlation coefficient of 0 81 with a probable error of 0 05 The correlation is sufficiently high to demonstrate a definite relationship between the ratios of lymph and serum

3 Two theories to account for the entrance of serum proteins into the lymphatics are discussed The first supposes general permeability of the entire capillary network of the blood for protein and leads to the conclusion that interstitial fluid is everywhere identical in composition with the lymph which drains the area The second assumes that occasional capillaries only permit the passage of protein, that this protein enters adjacent lymphatics at once, and that it never becomes disseminated throughout the intercellular spaces The available evidence, including the findings in this investigation, although consistent with either theory, does not justify the conclusion that interstitial fluid and lymph are identical

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PERMEABILITY OF CAPILLARIES TO PLASMA LIPOIDS

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It has been rather generally assumed, chiefly because normal urine is practically free from lipoids (9, 10, 11) that the capillaries are impermeable to plasma lipoids However, no serious attempts seem to have been made to test this assumption, in spite of its important bearing on the question of plasma colloid osmotic pressure In order to determine whether lipoids diffuse through the capillaries without simultaneous migration of proteins, two types of experiments have been conducted 1 Serum lipoids of normal individuals have been measured before and after the volume of the blood has been decreased, by a procedure which has been employed by Thompson, Thompson and Dailey (25), Krogh, Landis and Turner (13) and Ni and Rehberg (19) They demonstrated that if a normal person stands absolutely still for about half an hour the blood volume decreases and the serum proteins increase by about 10 per cent because there is transudation into the tissues of the lower extremities of a protein free ultrafiltrate of blood plasma 2 Besides, body fluids have been analyzed for lipoids and proteins

METHODS AND MATERIALS

The blood serum of five normal persons was analyzed for proteins, total fatty acids, lipoid phosphorus and cholesterol after they had reclined for half an hour and again after they had stood still for half an hour All the experiments except one were performed before breakfast Blood was drawn from the antecubital vein with a minimum of stasis under oil In all cases except that of P L the second blood sample was taken while the subject was standing In the second group of experiments, the protein and lipoid contents of ascitic, pericardial and pleural fluids were measured Blood and fluids were collected simultaneously, in most instances before breakfast All fluids were centrifuged to remove cells and fibrin clot from the aqueous suspension Proteins were determined by Howe's modification of the Kjeldahl method (5), total fatty acids by the Man and Gildea (16) modification of the Stoddard and Drury technique, cholesterol by a gravimetric digitonin method (17), lipoid phosphorus by a modification of the Fiske and Subbarow method (17)

RESULTS OF STANDING EXPERIMENTS

In Table I under each constituent the paired figures in the first

TABLE I

Standing experiments

Subject	Date			Lipoid P		Fatty acids from titer		Cholesterol		Proteins	
				mgm per cent	*change per cent*	*m Eq*	*change per cent*	*mgm per cent*	*change per cent*	*grams per cent*	*change per cent*
P L	January 11, 1933	Before		8 4	116 5	12 2	116 5	199	118 1	6 30	116 8
		After		9 8		14 3		235		7 47	
J H	January 23, 1933	Before		9 0	107 2	11 6	113 2	205	110 3	7 05	111 2
		After		9 6		13 1		226		7 84	
E M	January 3, 1933	Before		8 9	112 6	11 7	113 5	223	110 2	6 91	112 8
		After		10 0		13 2		246		7 83	
E M	February 17, 1933 *	Before		9 9	108 6	12 6	111 3	209	110 5	7 00	113 0
		After		10 8		14 0		231		7 91	
C R	January 28, 1933	Before		10 4	102 1	16 2	98 8	229	113 5	6 33	110 5
		After		10 7		16 0		260		6 99	
C R	February 28, 1933	Before		9 7	105 5	12 2	110 7			6 31	112 6
		After		10 2		13 5				7 10	
T K	February 1, 1933	Before		8 4	114 1	9 9	118 6	168	109 0	6 52	111 4
		After		9 6		11 7		183		7 27	

* Second blood taken four and one quarter hours after a breakfast which contained approximately 19 grams of fat

columns represent the values observed before and after standing, the single figures in the second columns the degree to which the given substance in the serum was concentrated The significance of "fatty acids from titer" is discussed more fully in the article describing the Man and Gildea modification of the Stoddard and Drury method (16) They found that saponification with potassium hydroxide as in the blood serum method, yielded only 82 per cent of the theoretical fatty acids from reprecipitated Pfanstiehl "pure" lecithin Consequently, in order to calculate "total fatty acids," there was added to the "fatty acids from titer" 18 per cent of the phospholipoid fatty acid estimated on the assumption that two equivalent weights of fatty acid unite with one combining weight of phosphorus in lecithin and cephalin Non-phospholipoid fatty acids may be calculated by subtracting from the "total fatty acids" the phospholipoid fatty acids estimated from the serum lipoid phosphorus content Values for "fatty acids from titer" have been presented here rather than those for non-phospholipoid fatty acids

because it is recognized that these calculations may introduce a considerable error if the analytical errors of the two methods are cumulative, and because neither the exact chemical composition of phosphatides, nor the proportions of different phosphatides present in serum are known

Cholesterol, without any exceptions, increased on standing in the same proportion as did the serum proteins, an indication that it is not diffusible   Fatty acids and lipoid phosphorus in 5 of the 7 experiments (P L , J H , E M , January 1 and February 17, and C R , February 28) increased in the same proportions as the proteins   On the other hand, in the serum of T K these constituents increased more than the proteins, and in the serum of C R on January 28 they did not change   These *divergent responses may be explained by the assumption that the* metabolism of fat of these subjects was not in a state of equilibrium   The change in T K 's blood sugar, from 76 mgm per cent to 85 after standing, suggests that the metabolism of carbohydrate was not uniform throughout the experiment   In contrast to the normal serum fatty acids in the first blood samples of P L , J H , and E M , the fatty acids both of C R on January 28 and of T K were at the extremes of the normal range of serum lipoids   C R 's total fatty acids on January 28 were the highest of 20 normal subjects and were appreciably greater than they were on February 28   After standing, his fatty acids and phospholipoids did not increase   T K 's serum fatty acids, on the other hand, which were among the lowest of the normal values, increased proportionately more than his proteins   From these data it may be inferred, though it is not proven, that C R on January 28 was utilizing fat derived from his blood stream, while T K was mobilizing fat into his blood stream   An attempt was made to test this hypothesis by carrying out a similar standing experiment on a subject after a meal containing fat However, the results of the experiment on E M on February 17 did not differ from those secured in the postabsorptive state, owing, perhaps, to the fact that too little fat was given

In these experiments, then, cholesterol without exception, phospha tides and compounds of fatty acids in five of seven instances, behaved like non diffusible serum proteins

Comparisons of transudates and serum

Data concerning the lipoid content of transudates are presented graphically in the three figures   In Figure 1 the *protein contents of* ascitic, pericardial and pleural fluids are plotted on the ordinate, cho lesterol content on the abscissa   The column above each point, at the top of the figure represents the concentration of cholesterol in the blood serum   The scale for serum cholesterol is 1/25 of that for transudate cholesterol   Irregular variations in the lengths of the columns show that the amounts of cholesterol in the fluid bear no direct relation to the
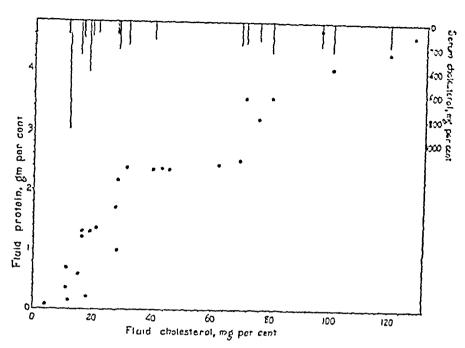
FIG 1   COMPARISON OF CHOLESTEROL AND PROTEIN IN TRANSUDATES
The vertical lines above indicate the concentrations of cholesterol in the sera of the patients from whom the transudates were secured
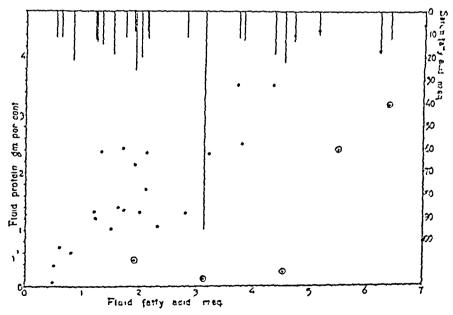
FIG 2   COMPARISON OF FATTY ACID AND PROTEIN IN TRANSUDATES
The vertical lines above indicate the concentrations of fatty acids in the sera of the patients from whom the transudates were secured   The points surrounded by circles are from patients whose sera contained little protein in proportion to fatty acids

concentrations of cholesterol in the serum   There is an obvious tendency for variations of protein and cholesterol contents of fluids to parallel one another

The arrangement of Figure 2 is similar to that of Figure 1, except that fatty acids from titer are plotted on the abscissa   As was the case with cholesterol, no correlation is evident between the concentrations of fatty acids in sera and transudates   A definite proportion exists between the protein and lipoid contents of the fluids except in those instances which are marked by circles

If the normal capillary wall is impermeable to lipoids and protein, injury, which increases its permeability, will permit the passage of both lipoids and proteins   The quantities of these substances which actually escape from the vessels will depend not only upon the degree of permeability to the individual components, but also upon the concentrations of these components in the serum   It has already been pointed out that the influence of the latter factor is not demonstrable in the data as a whole   It does, however, become evident in the points surrounded by circles, which represent instances when the protein in the serum was low in proportion to the lipoids   It is especially striking in the lowest of these points   In this instance the serum proteins were reduced to half the normal concentration, while the fatty acids were increased to about nine times the normal   Under these circumstances the lipoid concentration in the fluid might be expected to be, as it proved to be, higher in relation to the protein concentration than it was in the other observations in which the proportions of lipoid and protein in the serum were more nearly normal

In Figure 3, in which lipoid phosphorus and protein are compared, there is the same lack of correlation between the concentrations of the former in serum and fluid and the same direct relationship between transudate phosphatides and proteins, with one exception marked with a cross   The significance of this exception must be discounted because the peritoneal fluid, taken at autopsy from a patient with peritonitis, was semi purulent   Some of the lipoid phosphorus may, therefore, have come from pus cells and bacteria

DISCUSSION

It is obvious that the distribution of lipoids to the tissues by the blood stream must involve their passage through the capillary walls, in spite of the demonstrated impermeability of the latter   These experiments throw no light on the means by which this transportation is effected, whether the character of the lipoids is altered (3, 4, 22, 23, 24), or whether the impermeability of the capillaries is merely a facultative characteristic   In most of the standing experiments, during the half hour period, an equilibrium appears to have been reached and main
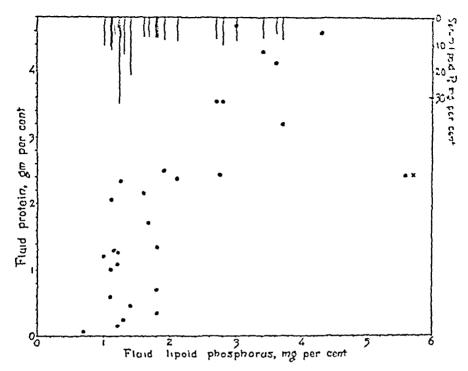
FIG 3   COMPARISON OF LIPOID PHOSPHORUS AND PROTEIN IN TRANSUDATES
The vertical lines above indicate the concentrations of fatty acids in the sera of the patients from whom the transudates were secured   The point marked by a cross represents peritoneal fluid secured *postmortem* from a patient with peritonitis

tained, in which the concentration of all lipoids, like that of proteins, was influenced only by the hemoconcentration that resulted from the loss of serum ultrafiltrate to the tissues   In two cases fatty acids and phospholipoids seem to have been simultaneously affected by some other influence, presumably connected with the metabolism of fat   That cholesterol was not similarly involved is only one more addition to the great body of evidence that this lipoid fraction is not directly concerned with fat metabolism   The problem of the exchange of lipoids between blood stream and tissue cells becomes even more mysterious when, from the studies of transudates, it becomes apparent how little lipoid material (8, 14) gains access through normally permeable vessels to the interstitial fluids, which presumably serve as the medium of exchange between blood and cells

The association between lipoids and proteins (2, 12, 15, 18, 20, 21, 29) in both types of experiment might suggest that the lipoids are restrained by combination with proteins   Such a deduction is not, however, obligatory   Such combinations may exist and the proteins may act as vehicles for a certain amount of lipoid material   In the case already

referred to in which protein was reduced and lipoid greatly increased in the serum, a greater amount of lipoid might have been expected in the transudates if it had been restrained from traversing the walls of the vessels only by its combination with protein

The exact correlation between the changes of cholesterol and protein in these experiments has a bearing on the nature of the interstitial fluids Thompson, Thompson and Dailey (25), comparing the volume of the blood, determined by the dye method, with the concentration of proteins in the serum, concluded that the fluid which passed from the blood stream into the tissues of the leg contained no protein   Waterfield (27), in similar experiments, in which he measured the blood volume by the carbon monoxide instead of the dye method, believed that he detected evidence of serum protein loss probably involving only the albumin fraction   More recently Youmans, Wells, Donley and Miller (30) have shown that the protein fractions and total proteins of the serum are all proportionately affected by the hemoconcentration   To these cholesterol can now be added   It is hardly conceivable that if the capillary membranes, under the conditions of these experiments, were permeable enough to permit appreciable quantities of protein to pass out, both fractions and cholesterol would all escape in exactly the same proportions   Certainly such an association is entirely incompatible with Waterfield's conclusions   Krogh, Landis and Turner (13) also found no evidence of loss of serum protein when transudation was induced in the arm by venous obstruction   It becomes harder in the light of these experiments to believe that capillary filtrates produced by venous stasis can contain as much protein as Drinker and Field (6) believe

If the capillary walls are impermeable to lipoids, the latter may exert an appreciable colloid osmotic pressure in the blood stream and, on this account, have some influence upon the exchange of fluids in the body Indirect evidence of various kinds indicates that the osmotic pressure of the lipoids is extremely small (1, 7 28), but more exact data on the subject are greatly to be desired

CONCLUSIONS

By standing experiments aimed to produce physiological transudation of fluid from the blood stream, and by comparisons of proteins and lipoids in serum and in pathological transudates, evidence has been adduced which indicates that the capillary walls are ordinarily impermeable not only to proteins but also to cholesterol, phosphatides, and compounds of saturated and slightly unsaturated fatty acids

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     Blood from the Foot in Relation to the Formation of Edema

THE CLINICAL SIGNIFICANCE OF THE ERYTHROCYTIC SEDIMENTATION TEST IN RHEUMATOID ARTHRITIS [1]

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Several investigators (1, 2, 3, 4) have called attention to the increased sedimentation rate of the red blood cells in patients with rheumatoid arthritis In order to ascertain the practical importance of this phenomenon, an extensive study was undertaken which continued for three years During this period, routine sedimentation tests were carried out on all patients with rheumatoid arthritis on admission to the arthritis department of the Cornell Clinic When possible, the test was repeated at varying intervals as long as a patient was under observation

Altogether 597 cases were included in this study, and the purpose of the following paper is to present an analysis of our findings

TECHNIC

The technic of the sedimentation test was similar in all respects to that of Rourke and Ernstene (5) and need not be described here The method of these investigators is particularly applicable in rheumatoid arthritis because of its correction for anemia which is a common though variable factor in this disease Heparin was used as an anticoagulant throughout the study and appeared to have no appreciable effect on the sedimentation rate The test was recorded as the corrected sedimentation index (C S I) and represents the rate of fall of the erythrocytes in millimeters per minute, after correcting for anemia Any result up to 0 4 was considered normal Unless otherwise stated, all sedimentation results reported refer to those at the time of the patient's first visit to the Clinic

Relation of the corrected sedimentation index to the activity of the disease

In this report, the term "activity of the disease" refers to the severity of the infectious process It must be recognized that this activity does not always parallel the degree of joint involvement as illustrated by "burned out" cases of arthritis in which swelling and deformity of the joints are present but the disease process is entirely quiescent

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In patients with rheumatoid arthritis, the activity, or severity, of the disease is often difficult to determine clinically It is roughly estimated from the amount of pain and tenderness in the joints, from the character rather than the degree of the swelling, and from the tendency of the process to migrate from joint to joint However, temperamental factors, which vary considerably in different patients and at different times in the same individual, frequently give rise to an entirely erroneous impression concerning the activity of the disease Weather and occupational conditions, likewise, play more or less important rôles in confusing the clinical picture In order to determine whether the difficulties caused by these variants could be eliminated by use of the sedimentation test, the results of this method when applied to a selected group of intelligent, mentally stable, and cooperative patients were analyzed The following protocols illustrate our findings

Case 1 H W , female, aged 45, admitted to the clinic March 16, 1928, gave a history of polyarthritis of six years duration At the time of admission, pain and stiffness of the joints were slight, and the patient was pursuing her usual occupation as a typist Physical examination revealed nothing of importance except in the joints—there was a slight but definite fusiform swelling of the fingers, with a low degree of swelling of the left knee and both ankles A diagnosis of mild rheumatoid arthritis was made During the succeeding three years, this patient was under our observation and throughout this period there was remarkably little change in her symptoms and physical condition The following results of the corrected sedimentation tests, taken over part of that time, closely paralleled the clinical impression January 1, 1931, 0 6, April 29, 1931, 0 55, September 25, 1931, 0 7, October 16, 1931, 0 6, February 29, 1932, 0 6

Case 2 J Mc , male, aged 20, admitted to the clinic on January 16, 1931, gave a history of polyarthritis of two years duration and complained of considerable pain at the time of admission Physical findings were unimportant except for the joint condition—the left hand and right knee were considerably swollen and slightly tender During the 15-month period that the patient was under observation, the feet, heels, shoulders, elbows, and right hand gradually became involved He consistently complained of much pain, and tenderness was frequently noted A diagnosis of rheumatoid arthritis was made It was considered a very active and rapidly progressing case, without any remission during the period of observation With this patient, the sedimentation rates closely paralleled the clinical impression, being high each time the test was made The results of these tests were as follows January 16, 1931, 1 7, May 1, 1931, 1 2, October 30, 1931, 1 6, November 25, 1931, 1 3, and March 16, 1932, 0 75

Case 3 N DeV , male, aged 46, was admitted to the clinic August 22, 1930, with a history of a rapidly progressing polyarthritis of nine months duration Physical examination revealed the typical fusiform swelling of the fingers, swollen knees and ankles, and stiffness of shoulders and hips Several of the joints were tender The remainder of the examination was irrelevant, except for the presence of a duodenal ulcer that caused no symptoms while the patient was under observation He showed no improvement while attending the clinic, and was admitted to the hospital January 2, 1931 A corrected sedi-

mentation test, carried out on December 29, 1930, a few days before admission, was 1 23   With rest in bed the patient made rapid progress toward recovery, and was discharged from the hospital on February 28 1931, as a cured case At that time he was entirely free from symptoms   There was, however, a small amount of swelling still remaining in the fingers and wrists, but this was considered as due to the scarring resulting from the disease, rather than as representing an active process   The corrected sedimentation tests during his stay in the hospital closely paralleled his clinical improvement, being 1 2 on January 2 1931 0 7 on February 7, 1931 and 0 4 on February 25, 1931—the figure recognized as normal in a corrected sedimentation test   Following his discharge from the hospital, the patient returned to work   On resuming his occupation, there was a marked return of the arthritic process   On May 19, 1931, his clinical condition approximated that on admission to the hospital four months previously, and his corrected sedimentation test was 1 6   During the succeeding year this case was followed in the out patient department, but there was no appreciable change noted   On April 29, 1932, when last seen, his arthritis was unimproved and his corrected sedimentation test was 1 9

Case 4   M O , female, aged 40, was admitted to the clinic March 14, 1930, with a three year history of pain and intermittent swelling in fingers and ankles Symptoms had never been severe, and she had been able to continue her house hold activities   Physical findings were unimportant except in respect to the joints   There was questionable swelling of the proximal interphalangeal joints and slight puffiness around the ankles   A diagnosis of mild rheumatoid arthritis was made   During the succeeding year she was observed in the clinic at irregular intervals, with little if any evidence of arthritis   On February 3, 1931, the date of her first test the corrected sedimentation index was 0 2

Beginning in March 1931, her arthritis gradually grew worse, and the fingers took on a marked fusiform swelling   In succession the knees, wrists, elbows, and shoulders became swollen and painful   There were aching pains in practically every joint in the body   The sedimentation test coincided perfectly with the change in her clinical condition, the index being 0 9 on April 15, 1931 and 1 3 on April 23, 1931   On May 11 1931 she again attended the clinic   At that time she was barely able to walk and her sedimentation index was 1 3   On May 23 of the same year, she made her last visit to the clinic and her index at that time was 1 1   Following this observation, she reported by telephone that she was unable to leave her bed

Case 5   J M   male, aged 24 admitted to the clinic on December 20, 1930, gave a history of a rapidly progressing polyarthritis of ten weeks duration Physical examination showed little of importance except for the joint condition There was fusiform swelling of several fingers, with swollen and tender knees and ankles   A diagnosis of rheumatoid arthritis was made and the condition was so severe that the patient was admitted immediately to the hospital   The sedimentation index on December 20, 1930, was 2 0   On the day of admission, the patient s temperature was 99 5° F , and was normal thereafter   With rest in bed and hospital care the arthritis rapidly improved from the day of admission   On February 13, 1931, the swelling had completely disappeared, and on February 25, twelve days later, the patient was discharged as free from signs and symptoms of the disease   The clinical change was correctly and immediately registered in his sedimentation tests   On December 29, 1930, the index was 0 65 January 13, 1931, it was 0 4 on February 7 and 25 of the same year it was 0 1 and 0 2, respectively

The first protocol is that of a patient running a mild course of arthritis throughout observation   Her sedimentation indices recorded at varying intervals during this time were remarkably consistent with the clinical findings, being slightly above normal each time the test was made   A similar relation between the sedimentation rate and the clinical findings was revealed in the second protocol, although the second patient had a severe process, and his sedimentation indices were consistently high

Protocols 3, 4, and 5 are of patients whose clinical picture varied markedly during observation   The third patient had a complete remission, the fourth developed a severe form of the disease from a very mild one, while the fifth recovered completely from a very active arthritis The sedimentation indices in each of these cases correctly and immediately registered the changes in the patient's physical condition

In the other patients of our series the relationship between the clinical picture and the sedimentation rate is similar to that in the five protocols given   Regardless of the fundamental factors underlying the sedimentation phenomenon, it is apparent that the erythrocytic sedimentation index parallels closely the severity, or activity, of the arthritic process

Relation of corrected sedimentation index to the degree of joint involvement

In the preceding section of our report, it was shown that the corrected sedimentation index is directly related to the activity, or severity, of the arthritic process   In the present section, a comparison of this index with the degree of joint involvement is considered   A differentiation between the activity, or severity, of the disease and the degree of joint involvement must be made, as it is recognized clinically that some patients suffering from a severe form of the disease have considerable pain, malaise, and even disability, with little change in the joints that may be detected by physical examination, whereas a patient may have marked swelling of the joints with deformity and ankylosis without the symptoms named above

In Table 1, the 597 patients studied are tabulated according to degree

TABLE 1

Relation of corrected sedimentation index to degree of joint involvement

Type of case	Number of cases	Lowest C.S.I	Highest C.S.I	Average C.S.I
Without joint swelling	308	0 1	2 0	0 64
With joint swelling	232	0 1	2 1	0 85
With swelling deformities and ankylosis	57	0 3	2 6	1 11
Total	597			

of joint involvement   Three hundred and eight of these had no appreciable joint changes at the time of examination, their sedimentation indices ranged from 0 1 to 2 0, with an average of 0 64   Two hundred and thirty-two of them had joint swelling without ankylosis, or deformities other than swelling   their sedimentation indices varied from 0 1 to 2 1, with an average of 0 85   Fifty seven of them were in the more advanced stages of the disease with deformities such as ulnar deviation and ankylosis, the sedimentation indices of this group ranged from 0 3 to 2 6, with an average of 1 11

From these results it is evident that, on the average, the greater the degree of swelling, deformity, and ankylosis, the higher the sedimentation rate, and, therefore, the greater the activity, or severity, of the disease, but as far as the individual patient is concerned, this deduction cannot always be made

Relation of the corrected sedimentation index to the age of the patient

In Table 2, the corrected sedimentation indices of the 597 cases are arranged according to the age of the patient   In this comparison, an interesting though unexpected relation was revealed   In patients in the third and fourth decades of life, a similar average sedimentation index was obtained but in those 40 years of age or over the average sedimentation rate showed a gradual increase all the way to the last group composed of patients 60 years of age or older

TABLE 2

Relation of corrected sedimentation index to age of patient

Age of patient	Number of cases	Average C S I
Under 30 years	69	0 69
From 30 to 39 years	122	0 68
From 40 to 49 years	181	0 73
From 50 to 59 years	170	0 83
60 years or over	55	0 96
Total	597	

The explanation of this gradual increase in the sedimentation rate with advancing years is not altogether clear   In the first place, it would seem probable that as patients grow older they tend to develop disease conditions other than arthritis which also affect the sedimentation rate   Our records indicate, however, that this factor plays no important rôle in the phenomenon, as no appreciable increase in disease unrelated to arthritis was detected in the older groups of our series   In the second place, it was observed that each succeeding decade is augmented by an increasing proportion of patients with severe arthritis who failed to recover from the disease earlier in life, as evidenced by an increase of the average disease duration with each succeeding age period   It seems

reasonable to conclude, therefore, that the increase in the average sedimentation rate with each succeeding age period is due primarily to the increase in the number of patients with severe arthritis, although other factors may play minor rôles in the results

Relation of corrected sedimentation index to season of year

Clinically it is generally recognized that in temperate climates patients with rheumatoid arthritis are usually at their worst in cold weather and at their best in hot weather   In order to determine whether this impression is confirmed by the sedimentation test, the indices of patients followed through the various seasons were analyzed   All patients on whom tests had been made in at least three seasons were included   With those having more than one test during any one seasonal period, the average was considered as that patient's index for that season   In the study, "Winter" covers the period from January 1 to May 31, "Spring" from April 1 to June 30, "Summer" from July 1 to September 30, and "Autumn" from October 1 to December 31

TABLE 3

Relation of corrected sedimentation index to season of year

Season of year	Number of Indices	Average C S I
Winter	58	0 865
Spring	62	0 840
Summer	38	0 799
Autumn	55	0 846

Altogether 69 patients were studied for seasonal variation   On these 69 patients, 58 tests were recorded in the winter, 62, in the spring, 38, in the summer, and 55, in the autumn   In Table 3, the average corrected sedimentation index for each of these recorded groups is presented   The highest figure occurred in winter, and the lowest, in summer, while spring and autumn gave intermediate results   The number of cases studied and the differences between the seasonal averages were too small to warrant definite conclusions   However, it is strongly suggestive that seasonal variations in the average sedimentation indices do occur, and as this test is an index of the activity, or severity, of the arthritis, that patients, on the average, are worse in the winter and better in the summer

Relation of the corrected sedimentation index to the duration of the disease

The records of the patients under observation were studied again to discover whether any relationship existed between the corrected sedimentation index and the duration of the disease   In the individual records little such relationship was found—some patients having had the disease for only a few weeks gave high sedimentation rates, while others having had it for many years gave a low sedimentation rate, and

vice versa In studying the average rates of sedimentation for various disease duration periods, however, it was found that there was a distinct tendency for these rates to increase as the duration of the disease increased (Table 4) From these results we conclude that patients having rheumatoid arthritis for a long period tend to have a more severe form of the disease than those having it for a short period, but that great individual variation exists

TABLE 4

Relation of corrected sedimentation index to duration of disease

Duration of disease	Number of cases	Average C.S.I
One month or under	9	0 70
Over 1 month to 3 months	50	0 71
Over 3 months to 6 months	63	0 70
Over 6 months to 1 year	87	0 77
Over 1 year to 3 years	120	0 76
Over 3 years to 5 years	74	0 79
Over 5 years	194	0 79
	597	

Relation of the corrected sedimentation index to the streptococcus agglutination test

In previous articles (6, 7), the authors showed that a high percentage of patients with rheumatoid arthritis gave a strong agglutination reaction with a biologically specific type of hemolytic streptococcus which, for convenience, they designated as "typical strain" This reaction appeared to be a true immunological response to a bacterial invader The question naturally arises as to what relationship, if any, exists between this agglutination reaction and the erythrocytic sedimentation rate An examination of the records of the cases in our series indicated that there was none whatever In patients with rheumatoid arthritis, the agglutinins are slow in developing and do not reach their maximum titer until about 6 months, on an average, has elapsed Following recovery of the patients, the agglutinins gradually diminish, but do not disappear, usually, until the patient has been free from the disease for several months, and sometimes years The sedimentation rate, on the other hand, is directly related to the activity, or severity, of the disease process, and changes directly with variations in this activity The difference in these two tests may be appreciated readily from a study of Figure 1, which is a graphic record of the agglutination and sedimentation tests of Case 5, previously described, and is typical of other similar records

DISCUSSION AND SUMMARY

The erythrocytic sedimentation test was performed on five hundred and ninety seven patients with rheumatoid arthritis in order to determine its significance in this disease
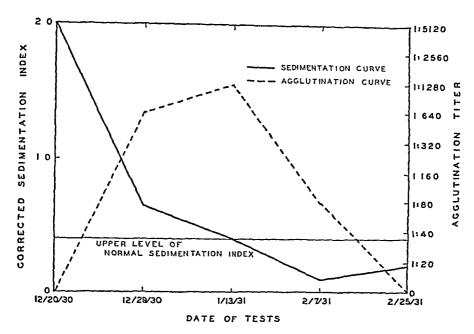
FIG 1   A COMPARISON OF THE RESULTS OF THE SEDIMENTATION AND
AGGLUTINATION TESTS OF CASE 5

The results of the investigation indicate that the corrected sedimentation index is a reliable criterion of the activity, or severity, of the arthritic process at the time of testing, and that any fundamental change in the clinical condition produces immediate corresponding change in the sedimentation rate

Patients with a greater degree of joint involvement and a longer disease-duration have higher sedimentation rates, on the average, than those with less joint involvement and shorter disease-duration   Considerable variation occurs, however, in individual cases

The observation that the average sedimentation rate progressively increases with advancing age periods is of interest   This phenomenon appears to be due primarily to the increasing accumulation of patients with severe arthritis in whom the disease began at some earlier age

From a study of seasonal variations in the sedimentation rate over a long period, suggestive evidence was deduced that, on the average, the rate was higher in winter than in summer, while spring and autumn occupied intermediate positions   Further work is necessary in order to confirm this impression

No relationship was found between the sedimentation rate and the streptococcus agglutination reaction

With the above information at hand, it seems justifiable to discuss the practical importance of this test   In the past, physicians treating arthritis have been greatly handicapped by lack of means for estimating

the results of their therapy   The sedimentation test appears to supply this widely felt need, as it is a reliable measuring rod of the activity, or severity, of the arthritic process   By repeating this test at regular intervals, the progress of the patient may be determined

Rheumatoid arthritis has attracted more than its share of ill conceived treatments   The sedimentation test provides a ready aid for correctly estimating the value of such procedures

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THE OXYGENATION OF CONCENTRATED VERSUS NORMAL BLOODS

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While the physiological activity of normal or dilute bloods represents one of the most widely studied fields of investigation, the problems presented by high concentrations of hemoglobin have not received the attention they merit   As far as the writers have been able to determine comparatively little attention has been paid to the ability of polycythemic bloods to take up oxygen under normal conditions   Barcroft and Murray (1) compared the effect of concentrating blood by centrifugating and removing part of the plasma   For a given O tension they found a higher degree of saturation in the concentrated blood than in normal blood which in turn took up more O_2 than did a sample diluted with the plasma which had been taken from the concentrated blood   This variation was considered secondary to a disturbance in the carbon-dioxide equilibrium   Richards and Strauss (2) found no difference between the dissociation curve of the blood of a polycythemic patient and that of blood from a normal individual

The experiments reported in this paper fall into two groups the first designed to repeat the tests of the oxygen combining power of concentrated blood *in vitro,* and the second to observe and compare the oxygenation of concentrated blood with that of normal blood in the perfused lung where the experimenter has a maximum control over the factors associated with oxygenation   Such a series of experiments should offer a concrete and definite answer to the question, does a plethora of red cells, *per se,* influence the respiratory function of the blood

Experiments on the O_2 combining power of concentrated blood

The technique employed in the handling of the blood and gases in these experiments was essentially that described by Austin et al (3) The concentrated blood was obtained by centrifugating blood of normal dogs and pipetting away most of the supernatant plasma   A measured amount of this blood, of known oxygen capacity, was placed in a small tonometer bearing at one end a two way stopcock and at the other a

tube of fairly large bore   The tubular end was connected to a similar
end of a large tonometer of the same type having a capacity of 250 to
300 cc   Care was taken to see that the blood filled the smaller tonometer
well into the rubber connection   This connection was then clamped and
the pair of tonometers connected with a manifold whereby one could
either evacuate the vessel or supply CO_2-free air, nitrogen, or carbon
dioxide   The tonometer was repeatedly evacuated and washed with
nitrogen after which sufficient CO_2-free air was admitted to give the
desired oxygen tension, following which the CO_2 tension was adjusted
to 40 millimeters   Pressure within the tonometer was then brought to
atmospheric by the admission of nitrogen   With the cocks closed the
tonometer was removed from the manifold and the clamp between the
blood and gas chamber taken off, allowing the blood to flow into the
larger chamber   In this manner portions of each sample of blood were
brought into equilibrium with gas mixtures of five different oxygen
tensions   The tonometers were rotated simultaneously in an air bath
at 37° C for thirty minutes   At the end of the period of equilibration
the blood was allowed to return to the small chamber and the clamp
replaced   The chambers were then disconnected, and samples taken for
analysis by forcing the blood into a pipette with mercury pressure
At no time was the blood allowed to come in contact with the room air
The analyses for oxygen content were made with the Van Slyke and
Neill constant volume manometric apparatus, oxygen capacity was
estimated by means of the spectrophotometer (Ray, Blair and Thomas
(4))   The final O_2 tensions existing in the tonometers were corrected
for oxygen lost from the blood and for the effect of temperature change
    The results of a characteristic series of experiments are given in
Figure 1   The open circles represent the results found in the normal
blood and the black those for the polycythemic blood   Both samples
were from the same original blood in order to avoid any chemical factors
which might influence the results   These data show beyond any doubt
that the oxygen-combining power of the two bloods is identical, under
the conditions of the experiments   In fact the distribution of the two
sets of points coincides quite as well as would points secured from two
samples of the same concentration   The variation of any point from
the mean curve is within the experimental error of the methods employed
    There is a point of distinction which can not be demonstrated in
the graph, but was definitely apparent during the course of the experi-
ment   The time required for the concentrated blood to reach an equi-
librium was markedly longer than that needed for normal blood   While
the normal sample showed its maximal color change in five to ten minutes
after the start of equilibration the concentrated needed almost double the
time for the same change   This was a natural sequence of the concen-
tration of the blood since diffusion is inversely proportional to viscosity
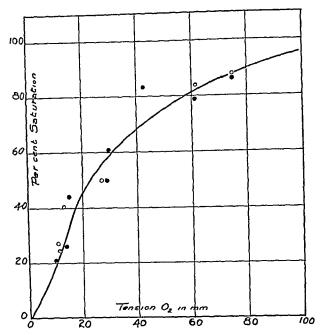
FIG 1   DISSOCIATION CURVE OF NORMAL AND POLYCYTHEMIC BLOODS
The open circles represent blood of normal hemoglobin concentration and the black, polycythemic blood

and also the amount of gas moved was greatly increased   The effect of both factors will be discussed in detail in a later section

Experiments with the perfused lung

The preceding experiments give no tangible evidence of a difference between the oxygen combining power of the two types of blood   Obviously if a difference does exist it must be sought in other directions We turned, therefore, to the perfused lung   These experiments were planned to determine if a normal or approximately normal oxygenating apparatus was capable of working with its usual efficiency when the number of cells passing through the tissue per unit of time was increased That is, if the rate of flow of hemoglobin through the lung is doubled can the respiratory conditions be so adapted as to produce a normal degree of oxygenation?   The importance of blood flow through a tissue

has often been stressed, but in this case it is, perhaps, preferable that attention be directed to the amount of hemoglobin as the essential factor

In order to study this effect we were forced to use a perfused lung Preliminary experiments on intact anesthetized dogs gave uncertain results   The results reported in this section were made on the isolated lungs of cats with the conditions of ventilation, blood flow, etc, made to simulate as closely as possible, normal conditions, while allowing full control over every variable   It was thus possible to study the respiratory function without the interference of the generalized compensatory mechanisms which were likely to be brought into play in the intact animal in response to a sudden massive injection of corpuscular cream

A diagram of the apparatus used in perfusing the lung is given in Figure 2   The lungs (1) and heart were removed as rapidly as possible from the anesthetized cat   In order to prevent clot formation during this process the animal was previously injected with heparin   A cannula (2) was inserted into the pulmonary artery by way of the right ventricle The tracheal cannula (3) was tied in   The outflow cannula (4) was inserted into the left auricle by way of the ventricle and the whole preparation placed in the jar (5) which served as a thorax   The metal plate (6) was clamped in place on a vaselined rubber ring, making an air-tight seal   The basic negative pressure was controlled by connecting tube (8) to a vacuum and expanding the lungs to approximately their normal size   This pressure was controlled by a manometer   The fluctuating negative pressure was produced by a pump (7) having a variable stroke and speed   A tambour connected at (9) recorded these fluctuations of pressure and so, upon calibration, served as a measure of the tidal air volume   This estimation was checked by a water manometer connected at (10) to the tracheal tube   The main branch of the tracheal tube went to a pair of mercury valves (11) of the type described by Bailey (5), which allowed passage of air with a minimum of resistance

For ventilating the lung atmospheric air enriched with approximately 5 per cent carbon dioxide was used, in order to maintain the acid-base relationships of the blood   This was stored in a 100 liter spirometer connected at (12) from which it was drawn into a smaller graduated spirometer (13) for use   The composition of the stored gas was checked from time to time to detect changes in O_2 and CO_2 content

,The blood used in these experiments was citrated ox blood   It is unfortunate that blood of another species had to be used, but the difficulties associated with collecting a sufficient amount of fresh cats' blood were insurmountable   Careful preliminary experiments were carried out to detect any indication of harmful reactions resulting from the above combination   Even after long periods of perfusion no deleterious effect could be seen other than the appearance of some edema which might have been expected, regardless of the nature of the perfusion fluid
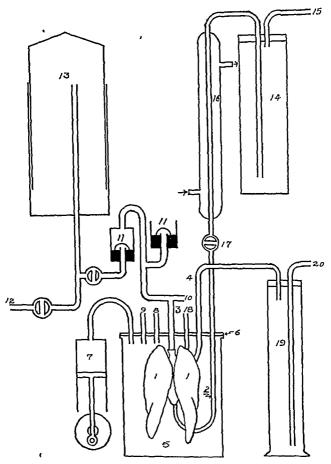
FIG 2   APPARATUS USED IN PERFUSING THE LUNG
Explanation of the figure is given in the text.

The fact that continued perfusions caused no striking changes in blood flow or ventilation was deemed evidence of the nontoxicity of the blood

The blood to be used in any particular series of experiments was divided in advance into two portions   One, the larger, was centrifugated and most of the supernatant plasma removed   Just previous to the experiment the sample to be used at that time was placed in a large bottle tightly stoppered with a two-hole rubber stopper, one opening of which was connected to a manifold by means of which vacuum, nitrogen and carbon dioxide could be controlled   The other opening of the bottle was tightly closed   The bottle was then evacuated and the resulting foam broken by the admission of nitrogen   This process was repeated until a vacuum produced no foam, which was taken as an indication that reduction was practically complete   In the case of concentrated bloods the process was, of course, much slower, but was accelerated by saturating the blood with carbon dioxide   The experiments might be criticized as unphysiological since blood of such low O_2 content was used, but it must be remembered that it was desired to test the ability of the lung to allow access of oxygen to the blood   Unless some common base line were adopted this estimation could not be made under exactly comparable conditions   The reduced bloods were kept under an atmosphere of nitrogen until they were ready to be introduced into the perfusion apparatus   At this time the sample was forced into the storage bottle of the apparatus (14) by pressure from a hydrogen tank and was kept under an atmosphere of this gas during the course of the experiment   The storage bottle was connected to an 18 liter bottle connected at (15), which in turn was connected to a constant water pressure   The blood was forced from the storage bottle through a condenser (16) to bring it to the proper temperature, and thence to the pulmonary artery   The pressure in this artery was adjusted by means of the stopcock (17)   A record of the pulmonary arterial pressure changes was recorded by a mercury manometer connected at (18)   The outflowing blood was caught in a 500 cc graduate (19) which served to measure the total blood flow while the rate of flow was computed from a tambour connected at (20)

Perhaps the feature to be most carefully avoided was the possibility of edema   In the preliminary experiments in which perfusion was carried out over a long period of time, edema was obviously present   In the course of these experiments certain signs were discovered whereby it was possible to recognize this condition at its onset and furthermore to estimate the duration of time that an experiment could be carried on without vitiating the results   Early edema was manifested by an increase in ventilation pressure and a decrease in venous pressure   No experiments in which these signs appeared are reported in this paper   In all experiments the period of perfusion was made as brief as was consistent with securing an adequate series of samples

The analyses for the degree of saturation were made on the Van Slyke apparatus, while the oxygen capacity was estimated from the hemoglobin concentration determined by means of the spectrophotometer Both arterial and venous samples were taken as nearly simultaneously as possible for each reading Any variation in hemoglobin content served as a check on fluid loss, and hence edema Obviously care had to be exerted that the storage bottle of blood was constantly agitated in order to avoid sedimentation In the first experiments we attempted to study both normal and polycythemic bloods in the same lung, but the period of perfusion extended over much too long a time and the practice had to be discontinued in favor of individual experiments for each type of blood

The general nature of the data obtained by this procedure may be illustrated in tabular form by a protocol of a pair of companion experiments

It will be noted that the degree of saturation of the venous blood in the case of the concentrated sample dropped markedly in the later

TABLE I

Protocol of a typical pair of experiments

Experiment	Time	Blood pressure		Intrathoracic pressure		Arterial blood			Venous blood		
		Arterial	Venous	Inspiration	Expiration	Oxygen capacity	Oxygen content	Per cent saturation	Oxygen capacity	Oxygen content	Per cent saturation
	minutes	*mm Hg*	*mm H₂O*	*mm H₂O*	*mm H₂O*	*volumes per cent*	*volumes per cent*		*volumes per cent*	*volumes per cent*	
A*	1 25	10	10	− 50	−20	15 34	4 32	28	14 74	14 83	100 0
	3 60	20	25	− 40	−10	15 27	4 08	26 8	15 14	15 13	100 0
	4 80	10	25	−100	−50	15 42	4 18	27 25	15 42	14 83	96 0
	7 50	40	45	− 60	−30	lost	4 08		15 42	14 90	96 5
B†	1 00	23	24	− 80	−40	39 25	1 42	3 60	37 59	26 82	71 4
	3 20	23	24	−110	−40	40 59	1 42	3 50	37 37	24 58	66 0
	6 50	23	24	−110	−40	39 39	1 42	3 60	38 83	21 88	56 3

* Experiment A Tidal air, 28 cc per respiration Rate of respiration, 40 per minute Lung capacity, 196 cc Average rate of blood flow, 35 cc per minute

† Experiment B Tidal air, 30 cc. per respiration Rate of respiration 20 per minute Lung capacity, 166 cc Average rate of blood flow, 40 cc, per minute

50

readings   As far as we could determine this was not due to any change in the lung tissue but rather to a continually diminishing tension of oxygen in the alveolar air resulting from the demands of the concentrated blood   Spectrophotometric evidence proved, in addition, that the hemoglobin had retained its ability to combine with oxygen   The slight variations noted in oxygen capacity are without doubt due to sedimentation, which could not be completely controlled

The results are summarized in Figure 3 in which the crosses represent the degree of saturation of the normal blood and the circles that of the concentrated blood   It will be noted that in every case the normal blood was oxygenated completely or almost completely, which is exactly what one would predict for the oxygen tensions used and the rate at which the blood was flowing through the lungs   The results indicate clearly the efficiency of the perfusion system
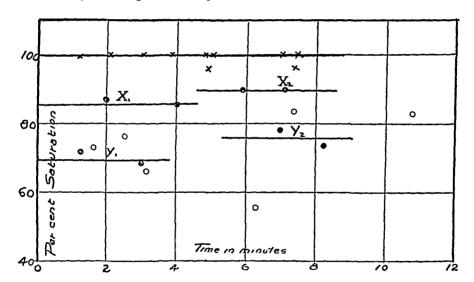
FIG 3   COMPARATIVE RESULTS OF PERFUSING THE LUNGS WITH NORMAL
AND POLYCYTHEMIC BLOOD

Crosses represent normal blood, the circles, polycythemic   Explanations of curves X_1, X_2, Y_1 and Y_2 are given in the text

In the concentrated blood on the other hand, a normal saturation was never attained   In spite of the fact that the lungs were to all intents and purposes normal, i e allowed oxygenation of normal blood, it is evident that with the usual ventilation and blood flow the system is incapable of functioning adequately   The incapacity of the system may be noted in a very simple manner   In two experiments, represented by curves X and Y, an attempt was made to reproduce what would be the normal compensatory reaction of an animal, i e the minute volume of respiration was increased   These results are of particular significance

since they show beyond question that the difficulty is not referable to any faults of the apparatus   In the routine experiments ventilation was within the normal resting limits defined by Scott (6) and by Churchill (7), whose observations agreed with our own measurements   It was further noted that hyperpnea, produced by stimulation of the sciatic previous to removing the heart and lung, raised the ventilation to about twice its resting value   Accordingly the minute volume was doubled in order to test the effect of a normal degree of hyperpnea

The ventilation in the section of the first curve, designated as X_1, was 700 cc per minute, which is within the normal range   At point X_2 the ventilation was increased to 1330 cc per minute   The increase in saturation is, however, far from proportional to the increase in ventilation, since the change is only from 87 to 90 per cent, which would correspond to a change in alveolar O_2 tension of about 5 millimeters

The results plotted in curve Y are more striking   The original ventilation, represented by section Y_1 of the curve, was 1200 cc per minute while in the section Y_2 the volume was increased to 2280 cc. per minute   In this case we have started with a moderate hyperpnea and increased the ventilation 190 per cent   In spite of this oxygen increase the saturation of the blood changes only from 70 to 77 per cent, equivalent, approximately, to 9 mm of O   It is obvious that if this represents the proportionality between ventilation and saturation, a tremendous hyperpnea would be required to produce a normal condition in the venous blood   It is conceivable that the oxygen saturation of the blood failed to increase under the unphysiological artificial conditions of these experiments because forced expiration did not have its usual effect, that no matter what the minute volume might be, the alveoli were incompletely aerated and therefore contained air with an unusually low oxygen tension   In order to test the validity of such a criticism the mode of attack was changed   Instead of increasing the amount of air sucked into the lungs at each respiration the amount of oxygen in the air was increased   Under these circumstances the problem of accumulation of nitrogen in the alveoli was eliminated and oxygen saturation depended only upon the rate of diffusion into the blood and subsequent oxygenation   In accordance with this scheme the ventilation gas mixture was changed to 94 per cent oxygen and 6 per cent carbon dioxide   The results proved definitely that even such a concentration of oxygen was barely adequate to approximate normal conditions, with this tension the average saturation was only 91 per cent   This figure indicates that it is about 6 to 7 times as difficult to oxygenate concentrated blood as it is to oxygenate normal blood

DISCUSSION

Certain definite facts stand out as the result of the above experiments   *A* With this method blood of normal concentration is adequately

oxygenated   *B* Increasing the number of cells, and consequently the amount of hemoglobin passing through the lung tissue per minute, results in inadequate oxygenation   *C* Doubling the amount of ventilation results in only a minor change in the saturation of concentrated blood   *D* Only when the oxygen tension of the inspired gas is increased to over six times that of the normal atmosphere does the saturation approximate normal

The question arises as to what physiological reactions are responsible for these differences between bloods of normal and high concentration The distinction appears to be inherent in the character of the blood and not referable to the conditions of the experiment, since normal blood behaves as it does when it is oxygenated in the physiological manner The lungs function efficiently as an oxygenating device   In fact, as the blood used was nearly completely reduced the tissue was called upon to do more work than would ordinarily be the case *in vivo*   The use of blood from another species seems to have caused no toxic effects   On the basis of these observations we are inclined, therefore, to dismiss the idea that there was any change in the lung tissue *per se*

The problem therefore resolves itself into a discussion of what factors in the blood or respiratory mechanism act, either independently or together, to produce the result found   The outstanding factor is perhaps the increased amount of hemoglobin passing through the lungs per minute   The demand for oxygen increases directly as the concentration of hemoglobin   In consequence, oxygen tension in the alveoli and the degree of oxygenation of the blood should diminish as the perfusion progresses   That this is the case is illustrated by the experiment shown in Experiment B, Table I, where the saturation decreased from 71 to 56 per cent during the period of measurement

This might afford an adequate explanation of the whole phenomenon, were it not for the fact that increased ventilation of the lungs proved insufficient to produce normal oxygenation and also that it was found necessary to increase the oxygen tension to seven times that of normal before full saturation was even approximated   In the case of the experiments in which the ventilation was increased, the change in the degree of saturation was by no means proportional to the increase in the minute volume of respiration   The increased movement of air failed to change the tension of alveolar oxygen to any marked extent   Perhaps more significant is the high pressure necessary to drive a sufficient amount of oxygen into the blood   In other words, the rate of diffusion of oxygen is reduced in the case of the more concentrated blood   This is exactly what Harrop and Heath (8) found in polycythemic individuals   These investigators ascribe this change to alteration of the pulmonary epithelium   This cannot be said to be true in our experiments, inasmuch as the lungs behaved in a normal manner when perfused with blood

containing a low concentration of cells It is obvious, therefore, that the reason for the faulty oxygenation of the blood must be sought elsewhere It will be recalled that in the experiments upon the dissociation curves a longer time was needed for complete equilibration of the concentrated blood It would seem, therefore, that a certain fraction of the unsaturation can be attributed to the character of the concentrated blood itself

Still another factor to be considered is the reaction which occurs in the capillaries In the table cited it will be noted that the amount of blood flowing through the system is approximately the same in both experiments The viscosity of the concentrated blood is much greater than normal, yet if the second readings of both types of experiments are compared it will be noted that the same pressure produces the same flow The simplest explanation that can be suggested for this adjustment is dilatation of the smaller vessels The appearance of the lungs in the two cases certainly substantiated this view If such a compensation involved the alveolar capillaries a second reason for the impairment of oxygen diffusion is found in the increased distance the gas must pass in order to complete the process of oxygenation In addition to this effect, the engorgement of the vessels would decrease the alveolar space, i e , the vital capacity, thereby adding to the factors which promote anoxemia

SUMMARY

Studies of the dissociation curves of normal and artificial polycythemic bloods, show no difference in the tension of oxygen required to produce a given saturation When these types of blood are oxygenated by the perfused lung the normal blood becomes completely oxygenated while the concentrated blood is never fully saturated Increasing the oxygen tension increases the saturation of the concentrated blood

The difference between the two bloods is ascribed to the greater rate at which hemoglobin in the polycythemic blood passes through the lungs, coupled with a delayed diffusion resulting from capillary dilatation

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ELECTROLYTES IN HUMAN TISSUE III A COMPARISON OF NORMAL HEARTS WITH HEARTS SHOWING CONGESTIVE HEART FAILURE [1]

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Previous studies from Vanderbilt Medical School (Harrison, Pilcher and Ewing, 1930) (Calhoun, Cullen, Clark, and Harrison, 1930) have shown that the potassium content of heart muscle may be diminished in persons dying with congestive heart failure The present study was planned to investigate the concentration of other important electrolytes under the same conditions and in normal hearts, i e , hearts from persons killed in accidents However, when the pathologic reports were analysed it was found necessary to divide the material into three groups—(1) Normal hearts, (2) an intermediate group of hearts from fatal accidents which show pathologic evidence of cardiac disease, and (3) hearts from persons dying with congestive heart failure See Table I

Seventeen hearts, 5 in Group 1, 4 in Group 2, and 8 in Group 3, were analysed for water content, calcium, magnesium, phosphorus, potassium and sodium

The methods used in this study are those described elsewhere (Cullen and Wilkins, 1933) Special precautions were taken to determine accurately the water content of the tissues The samples, which were shipped, were sealed in air tight bottles, and any liquid which came out of the tissues during transit was included in the entire sample which was weighed and dried

RESULTS

The determined values, in most cases based on duplicate analyses, for water, total phosphorus, potassium, sodium, calcium and magnesium are given in terms of wet muscle in Table II In order to compare the osmotic relationship of the electrolytes in the tissue, the results were calculated to milliequivalents per kilo tissue water, as was done by Van Slyke, Wu and McLean (1923) for blood electrolytes In this

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TABLE I

Individual case data

Case number	Sex	Age	Heart weight	Chief diagnosis and cause of death
		years	*grams*	
Group 1				
1	M	34		Bullet wound of abdomen, hemorrhage, heart normal
2	M	24	400	Bullet wound of head, heart normal
3	M	25	265	Fracture of skull, ruptured liver and kidney, heart normal
4	M	33	400	Bullet wound through lungs and heart, heart *normal*
5	M	25		Bullet wound of abdomen, hemorrhage, heart normal
Group 2				
6	M	38	535	Fracture of skull
7	M	31		Bullet wound of abdomen, hemorrhage
8	M	49	450	Lacerations of head, dislocation of hip, (chloroform anesthesia) toxic hepatitis, myocardial hypertrophy, generalized arteriosclerosis
9	M		400	Multiple comminuted fractures of the pelvis, hemorrhage, alcoholic intoxication, generalized arteriosclerosis
Group 3				
10	M	25		Myocardial hypertrophy and dilatation, adhesive pericarditis
11	M	33	770	Myocardial hypertrophy and dilatation, syphilitic aortitis and insufficiency
12	F	23	450	Myocardial hypertrophy and dilatation, syphilitic myocarditis
13	M	72	400	Myocardial hypertrophy, generalized arteriosclerosis
14	M	40	860	Myocardial hypertrophy and dilatation, generalized arteriosclerosis
15	F	45	740	Myocardial hypertrophy and dilatation, generalized arteriosclerosis
16	M	69	550	Myocardial hypertrophy and dilatation, generalized arteriosclerosis
17	M	55	800	Myocardial hypertrophy and dilatation, generalized arteriosclerosis

calculation one millimol of phosphorus is considered one milliequivalent (see Peters and Van Slyke (1931), p 1102) The average, maximum and minimum values for the several elements are summarized in Table III In addition, in this table, is given a similar summary based on dry weight because much of the previous work has been reported in dry weight

Water

Domagk (1924) and Scott (1931-*a*) have shown that the water content of the left ventricles of persons who died of tuberculosis was greater than that of the left ventricles of persons dying from a variety of other diseases Apparently neither of these workers determined the water content of the right ventricles Scott (1930, 1931-*a*) found no difference in the average water content of the left ventricles of cardiac heart and that from a number of persons who died from other diseases

TABLE II

Inorganic constituents of ventricles of normal and diseased hearts

Case number	Right ventricle						Left ventricle					
	Per 100 grams wet tissue											
	H₂O grams	P mgm	K mgm	Na mgm	Ca mgm	Mg mgm	H₂O grams	P mgm	K mgm	Na mgm	Ca mgm	Mg mgm
	H_2O grams	P mgm	K mgm	Na mgm	Ca mgm	Mg mgm	H_2O grams	P mgm	K mgm	Na mgm	Ca mgm	Mg mgm
Normals												
1	80 1	203	229	96	8 2	21 8	78 1	242	324	73	6 6	23 2
2	80 4	164	259	133	9 3	18 2	79 5	184	280	114	9 5	18 8
3	76 9	180	285	111	7 5	20 3	78 0	199	325	98	6 3	20 4
4	81 2	142	220	112	7 0	16 4	80 1	179	302	97	6 2	15 6
5	77 6	195	280	83	5 9	22 3	78 8	213	326	76	6 0	22 6
Average	*79 2*	*177*	*255*	*107*	*7 6*	*19 8*	*78 9*	*203*	*311*	*92*	*6 9*	*20 1*
Intermediate												
6	80 9	137	205	148	8 2	15 0	79 8	177	262	118	6 3	18 6
7	83 7	159	201	181	6 2	14 6	80 9	213	332	106	6 4	20 2
8	83 0	146	178	130	9 2	15 7	80 3	154	217	81	6 4	16 5
9	79 0	146	203	138	9 4	15 8	78 9	197	326	101	6 9	19 4
Average	*81 7*	*147*	*197*	*149*	*8 3*	*15 3*	*80 0*	*185*	*284*	*102*	*6 5*	*18 7*
Cardiacs												
10	82 0	143	223	129	6 4	15 1	82 1	149	237	128	5 6	15 4
11	82 3	152	209	155	5 4	13 8	80 4	178	226	115	4 4	15 7
12	83 6	122	134	186	8 9	11 6	81 9	142	209	154	6 7	14 0
13	82 9	150	174	153	9 9	14 6	81 5	176	236	132	10 9	15 6
14	82 4	142	180	147	7 2		80 4	173	277	92	5 6	
15	81 4	145	181	148	5 5		79 1	170	248	102	5 0	
16	78 2	158	235	120	8 7	14 6	78 9	185	325	94	7 1	18 0
17	79 3	183	265	101	4 5	18 5	79 4	188	303	100	4 4	16 3
Average	*81.5*	*149*	*200*	*142*	*7 1*	*14 7*	*80 5*	*170*	*258*	*115*	*6.2*	*15 8*

In the present study there is no evidence from the 5 normal hearts of a consistent variation in water content between right and left ventricles, but for the diseased group there is a definite tendency for each right ventricle to have a higher water content, by about 1 per cent, than that of the left from the same heart Moreover, the heart from persons dying with congestive heart failure showed an average of about 2 3 per cent more water in the right ventricles and about 1 6 per cent more in the left ventricles than did the normal hearts These changes stand out more emphatically when stated in terms of percentage solids This

TABLE III

Averages of inorganic constituents of ventricles calculated on basis of dry-weight and of tissue water

Group	Mgm per 100 grams dry tissue		m Eq per kilo tissue H O	
	Right ventricle	Left ventricle	Right ventricle	Left ventricle
Phosphorus				
Normal average	852	962	72	83
Intermediate average	811	926	58	74
Cardiac average	808	870	59	68
Potassium				
Normal average	1225	1475	83	100
Intermediate average	1081	1422	61	91
Cardiac average	1075	1314	63	82
Sodium				
Normal average	521	436	58	51
Intermediate average	828	508	79	55
Cardiac average	786	594	76	62
Calcium				
Normal average	36 9	32 7	4 8	4 4
Intermediate average	45 0	29 9	5 1	4 1
Cardiac average	38 8	33 4	4 3	3 9
Magnesium				
Normal average	95 4	95 0	20 6	21 0
Intermediate average	84 0	93 5	15 4	19 2
Cardiac average	79 0	82 0	14 9	16 2

means a decrease from normal of 11 per cent of the total solids in the right ventricles and of 7 5 per cent in the left ventricles   Apparently the right ventricle shows a greater susceptibility to increase in water content or decrease in solids than does the left ventricle

This is in agreement with the findings of Calhoun, Cullen, Clarke and Harrison (1930) who found that right and left ventricles in patients who died with congestive heart failure showed an increase in water content over the hearts of patients who died from a variety of other diseases It is also of interest to note that the two right ventricles which contained the most water were also high in sodium   Of these, one showed also the lowest potassium content on any basis of calculation   This right

ventricle (case number 12), was also the lowest in magnesium and phosphorus per unit of wet weight   These facts indicate a definite tendency toward edema in diseased heart muscle, especially that of the right ventricle

Phosphorus

The figures for phosphorus represent total phosphorus in each case, no attempt was made to determine the phosphorus fractions

Scott (1931 a) reported a large series of phosphorus determinations on the left ventricles of persons who died with a variety of diseases and a group who died of tuberculosis   The following is a summary of his values

	Maximum	Minimum	Mean
	mgm per 100 grams of fresh tissue		
General diseases	233	139	176
Tuberculosis	193	129	153

In the present normal group the left ventricles contained an average of about 15 per cent more phosphorus than did the right   In the group of persons who died with congestive heart failure the left ventricles contained an average of about 14 per cent more phosphorus than the right   Both the right and left ventricles of the persons who died with congestive heart failure contained an average of about 16 per cent less phosphorus than did the corresponding ventricles of the normal group These findings are in agreement with those of Laszlo (1928) who noted a decreased amount of phosphorus in the skeletal and cardiac muscles of persons who died with cardiac disease

Potassium

In this study the individual left ventricles of each group showed without exception a higher concentration of potassium than did the right This contrasts with the fact that the left ventricles of each group without exception showed a lower content of sodium than did the right   One case with extreme congestion of the lungs showed very low values for potassium and high values for sodium in both ventricles, but especially in the right

When these values are expressed in milliequivalents per kilo of muscle water, the total concentration of sodium plus potassium in the right ventricles is of the same order as that of the left ventricles

The hearts of individuals who died with congestive heart failure were poorer in potassium and richer in sodium than those of the normal control group   The figures show that there was some degree of overlapping, but the averages show a definite and appreciable difference

This is true when the concentrations are expressed in milligrams per 100 grams of fresh tissue, milligrams per 100 grams of dried tissue, and in milliequivalents per kilo of tissue water

These findings, in respect to potassium, confirm, with a different analytical method, those of Harrison, Pilcher and Ewing (1930), and those of Calhoun, Cullen, Clarke and Harrison (1930)   The latter authors believe that overwork causes loss of potassium from cardiac muscle and that this loss is one of the predisposing factors to cardiac fatigue and failure   They also studied a number of different tissues from individuals who died of a variety of diseases, and suggest that potassium loss in congestive heart failure is not confined to the heart and skeletal muscle, but apparently holds for several other types of tissue   Later Calhoun, Cullen and Harrison (1930) found that overwork of the muscles of one leg of dogs, produced by continual stimulation of the sciatic nerve causing the leg to lift a weight, usually leads to a diminished content of potassium in the muscles as compared with those of the opposite un-stimulated leg

Harrison, Pilcher, and Ewing (1930) suggested the possibility that loss of potassium from the heart muscle *is compensated for by an increase* in one or more of the other basic elements   The present study shows that there is a "compensation" by sodium

Scott (1931-*b*) who has made the most extensive study of the in-organic substances in human left ventricle heart muscle, failed to find a significant diminution of potassium in the left ventricles of persons who died of congestive heart failure compared with the left ventricles of persons who died from a variety of other diseases   He found highly variable amounts of both sodium and potassium in both groups   His average values for potassium in the left ventricle are much lower than those of other authors, including ourselves

The discrepancy between Scott's values (1931-*b*) for potassium and the present results has been disturbing   The possibility that this is due to a difference in ashing is suggested by the following observations   When the present investigation was begun, a number of tissues were ashed in somewhat the same manner as were those of Scott, and results for potassium similar to his were obtained   These results were low as compared with the previous studies in this laboratory, and the ashing technic was scrutinized   It was found that if ashing were done in the muffle furnace without H_2SO_4, the potassium values were low   When a small amount of H_2SO_4 was added before ashing the duplicate ashings gave potassium figures which were higher, which checked closely, and which were within the range of those previously obtained when the wet ashing technic was used   Blanks were, of course, run under both sets of conditions

In a more recent study with an improved method Scott (1931-*c*) reported much higher values for potassium in the left ventricles of a large number of hearts than he reported in the three previous studies   These later values are more nearly within the range of ours

Sodium

Few figures are available on the sodium content of the various soft tissues, although its concentration in the blood has been the object of numerous investigations

In the human, sodium appears to be present in much greater concentrations in the extracellular fluids than in any of the cells In this respect it differs from potassium and magnesium, which appear in greater concentrations in the cellular structures Of the anions, chloride appears to vary with sodium, while phosphate varies with potassium

Loeb, Atchley and Palmer (1922) have shown that edema fluids may contain approximately the same concentration of sodium as blood serum It follows that even a slight increase in the water content of a tissue may be accompanied by an appreciable increase in sodium

In the present study the sodium content of the right and left ventricles of seventeen human hearts was determined In all seventeen cases, with two exceptions, the right ventricles contained decidedly more sodium than did the left ventricles In every instance, the opposite was true of potassium

In both ventricles the normal hearts showed a higher average content of potassium than did the corresponding ventricles of persons who died with congestive heart failure Just the reverse was the case with sodium There was some overlapping with reference to sodium as well as with potassium, but most of the individual cases, as well as the averages, showed a definite increase in the sodium content of both ventricles of the persons who died with congestive heart failure

The four left ventricles which had the highest potassium content showed the lowest values for sodium

Scott (1930) reported a number of instances in which the normal K/Na ratios were almost reversed in the left ventricle We found some tendency toward such a reversal in both ventricles of several persons who died with congestive heart failure, but found no such change in any heart in the normal group

Scott's averages for the sodium content of the left ventricles are higher than those yielded by the present study In view of the fact that his values for sodium were calculated by difference, his higher figures for this element may have been due to his failure to recover all of the potassium This point has been discussed in the section on potassium

Calcium and magnesium

Although the significance of changes in calcium concentration in the blood has been studied intensively little is known concerning changes of calcium in tissue Little is known concerning the importance of magnesium in either blood or tissue Magnesium is essentially a tissue cation, calcium is in greater concentration in serum and other extracellular

fluids than it is in most soft tissues   Katz showed that human skeletal muscle contained about 7 5 mgm per cent of calcium and 21 mgm of magnesium per 100 grams fresh muscle   On a basis of milliequivalents this gives a Ca   Mg ratio of about 1–4   Scott found in the left ventricles of 14 hearts from patients with general disease an average of 8 mgm of calcium and 17 4 mgm of magnesium per 100 grams fresh tissue and in 14 hearts from patients dying with tuberculosis 9 0 mgm calcium and 16 mgm magnesium

In another study (Cullen, Wilkins and Harrison (1933)) we found in autopsies from general disease that the right ventricles average 7 3 mgm per cent of calcium and 18 4 mgm per cent magnesium, and the left ventricles averaged 6 4 mgm per cent calcium and 21 mgm per cent magnesium   In the present study 15 of the 17 hearts show slightly less calcium and more magnesium in the left ventricle than in the right

The variation in calcium content in both normal and diseased hearts is so great that, with the small number of cases, it is impossible to state whether or not there is less calcium in the diseased heart than in the normal   There is a tendency for the abnormal hearts to show a decrease in calcium proportional to the decrease in total solids which suggests that the calcium is related to the tissue protein in the same manner that it is related to serum protein   Scott, however, found no significant difference between the calcium content of the left ventricles of patients dying of heart failure and of those dying from other diseases

In most cases the hearts showing cardiac disease have a decidedly lower magnesium content than do the normal hearts

In general the variations of magnesium content parallel those of potassium and phosphorus

GENERAL DISCUSSION

Total base determinations, as such, were not made on this group of tissues, but were calculated from the values for the individual bases We found, in a former study, that some calcium and magnesium failed to come through during the removal of phosphate by the method of Stadie and Ross (1925)   This fact has also been noted by Brown and Shohl (1931)

In all of the normal group the concentration of total bases (in milliequivalents per kilo of tissue water) was higher in the left ventricle than in the right, but both were within the same general range

The right ventricles of the normal hearts show an average of 167 and the left ventricles an average of 176 milliequivalents per kilo of tissue water   The hearts from persons who died with congestive heart failure contained an average of 159 milliequivalents per kilo of tissue water in the right ventricles and 166 in the left   Thus the latter group showed in both ventricles a slight diminution in total base as compared with the normal controls

Van Slyke Wu and McLean (1923) have pointed out that the osmolar concentration of total electrolytes in the water of blood plasma and cells is practically equal despite the differences in the concentrations of the individual ions   They found, in a typical case, 162 milliequivalents of base per kilo of serum water and 174 per kilo of red blood cell water Gamble, Ross and Tisdall (1923) have expressed the belief that this close relationship holds also for the cell water of muscles and probably for other tissues of the body   The present results support this view

The total electrolyte concentration in the tissue is of importance in relation to the osmotic conditions existing there   Although the data given above do not give any direct evidence as to the cause of the shift in diseased tissue, it is significant that it is precisely what would be expected if the permeability of the tissue were increased   Then sodium would diffuse into the tissue cells from its higher concentration in extracellular fluids, and potassium, magnesium and phosphates would diffuse out from their higher concentration in the cells   Calcium, approximately equal in all fluids, would show little change   The relations between potassium, phosphorus and sodium are shown graphically in Figure 1 It is at once apparent that potassium and phosphorus parallel each other and that sodium fluctuates regularly in the opposite direction to potassium and phosphorus

The question of the ratio of the individual elements to each other is of course important and the average ratios of P/K and K/Na are given in Table IV

TABLE IV

Ratio of potassium to phosphorus and sodium based on concentration per kilo tissue water

	Millimols P / Millimols K		Milliequivalents K / Milliequivalents Na	
	Right ventricle	Left ventricle	Right ventricle	Left ventricle
Normal average	0 88	0 83	1 45	2 05
Intermediate average	0 96	0 82	0 78	1 65
Cardiac average	0 96	0 84	0 88	1 39

The data demonstrates unmistakably a real difference in the concentration of the important electrolytes in the two ventricles   It is important that both potassium and phosphorus, which are known to be involved in the buffer mechanism of muscle are in the muscle which has the greatest work load   However, until the phosphorus distribution in these conditions is known, it is undesirable to speculate further

Averages for P, K, and Na

FIG 1
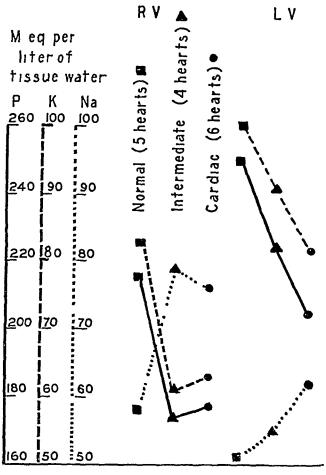
Muehlberger of the Coroner's Laboratory, Cook County Morgue, Chicago, and to the Department of Pathology of the Vanderbilt School of Medicine, for furnishing us the tissues used in this study

SUMMARY

1 The right and left ventricles of seventeen human hearts were analyzed for water, phosphorus, sodium, potassium, magnesium and calcium Of these hearts, five were normal, four were from persons who had cardiac disease but who died from other causes, and eight were from persons who died with congestive heart failure

2 The water content of the diseased right ventricle tends to be slightly higher than that of the left ventricle The water content of both ventricles of hearts from persons who died with congestive heart failure was found to be increased

3 The normal left ventricle contains more total phosphorus and more potassium than the right   Both ventricles of diseased hearts showed a decrease in total phosphorus and potassium

4 The normal right ventricle contains more sodium than the normal left ventricle   Sodium was increased in both ventricles of persons who died with congestive heart failure

5 Usually the right ventricle contains a slightly higher concentration of calcium than does the left ventricle   No consistent variations were found in the calcium content of the ventricles of individuals who died with congestive heart failure

6 Both the normal and diseased left ventricles were richer in magnesium than the corresponding right ventricles   Both ventricles of the diseased hearts showed a diminution in magnesium

7 The sums of the individual bases when calculated in milliequivalents per kilo of tissue water show that the two ventricles do not differ essentially in their content of total base

8 The K/Na ratio in the normal left ventricle is higher than in the normal right ventricle   Both ventricles of the diseased hearts showed a decrease in the K/Na ratio

9 The P/K ratio was somewhat higher in the right ventricle than in the left   The diseased hearts had nearly the same P/K ratios in both ventricles as did the normal hearts, showing that the former had a proportionate decrease in phosphorus and potassium

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THE CISTERNAL PRESSURE IN CONGESTIVE HEART FAILURE AND ITS BEARING ON ORTHOPNEA

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Various theories concerning the cause of orthopnea have been advanced, but sufficient proof for accepting any one explanation of this phenomenon has not been offered

As an excellent review of the literature on orthopnea has been given by Ernstene and Blumgart (1), only a few of the theories will be mentioned here   Sahli (2) thought that the greater use of the accessory muscles of respiration, the accumulation of blood in the lower extremities leading to diminished pulmonary congestion, and a decrease in the work of the heart along with the relief of venous congestion of the brain and of the respiratory center by the action of gravity were the fundamental factors responsible for the sitting posture in orthopnea   Haldane, Meakins and Priestly (3) expressed the belief that a decrease in anoxemia of the respiratory center and a better oxygenation of the arterial blood leaving the lungs were largely responsible for the greater comfort in breathing that a patient with cardiac disease exhibits in the upright position

Christie and Beams (4) reported careful studies of the vital capacity of a large number of normal subjects and of patients with cardiac disease They found that, although the vital capacity was decreased when the normal person went from sitting to prone position, this decrease was much greater in patients with cardiac disease accompanied by orthopnea They, therefore, inferred that the diminished vital capacity of the lungs in the prone position is the main cause of orthopnea

Ernstene and Blumgart (1), however, observed *orthopnea of necessity* in patients in whom the vital capacity of the lungs was not significantly increased by changing from the recumbent to the sitting position   They demonstrated furthermore a parallelism between the height of venous pressure and the degree of orthopnea in twenty two patients with uncomplicated myocardial failure   They were inclined to the view that lowering of cerebral venous pressure in the sitting posture is the primary factor in reducing the respiratory discomfort

In a recent series of studies Harrison (5) demonstrated an increase in cerebrospinal fluid pressure in patients with cardiac failure and the parallelism of venous and spinal fluid pressure in this condition   He

also observed the effect of treating by means of spinal drainage patients having congestive heart failure It was noted in observations not yet published that after spinal drainage was performed on individuals with orthopnea many of these patients were able to breathe more comfortably in the recumbent position, although the vital capacity of the lungs had not been significantly altered by this procedure A study was also made of the cisternal pressure in the upright and recumbent positions in one normal individual, and in one patient with congestive heart failure, and the relation of cisternal pressure to orthopnea was discussed In the present paper a larger group of such studies is reported

The cisternal pressure in patients without cardiac disease

While performing routine lumbar punctures on patients suspected of having syphilis an opportunity was offered to measure the cisternal pressure and the spinal fluid pressure in the sitting and prone positions in five patients, none of whom had any evidence of cardiac disease A U-shaped glass manometer filled with normal saline was used to make the readings of cisternal pressure The readings of lumbar spinal fluid pressure were taken with an Ayer water manometer Venous pressures were determined by the direct method of Moritz and Tabora observing the usual precautions The vital capacity, the arterial blood pressure, and the pulse and respiration rates were likewise recorded in both positions (Table 1)

The systemic venous pressure, as measured at the elbow, was found to be slightly higher in the upright than in the recumbent position The lumbar spinal fluid pressure was much greater in the sitting position The cisternal pressure (which ranged from − 22 to + 17) was on the other hand much lower in the sitting position, and in one individual was below zero The average arterial blood pressure and pulse rate were slightly greater in the sitting position, but neither the blood pressure nor pulse or respiration rates showed marked consistent changes, on the other hand in all instances the vital capacity was slightly increased in the sitting position

The cisternal pressure in patients with congestive heart failure

An opportunity was offered to carry out similar observations on five patients who had either syphilitic aortic insufficiency or hypertensive heart disease with congestive failure, while attempts were being made to give relief by spinal drainage Similar measurements were made in the manner described above All observations on any one patient were performed on the same day [1] (Table 2)

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[1] The cisternal pressure recorded was slightly lower in some instances and the spinal fluid pressure lower in others than they should have been because in some cases the spinal fluid pressure was taken first while in others the cisternal pressure was measured first

TABLE 1

Control group

	Venous pressure		Spinal fluid pressure		Cisternal pressure †		Vital capacity		Arterial blood pressure		Pulse		Respirations	
	P*	S*	P	S	P	S	P	S	P	S	P	S	P	S
	mm H₂O	mm H₂O	mm H₂O	mm H₂O	mm H₂O	mm H₂O	cc	cc	mm Hg	mm Hg				
J R Syphilis	65	100	60	350	125	10	2800	2900	108/90	130/100	72	76	24	20
T H Catarrhal jaundice	50	70	125	430	102	15	1900	2000	100/64	110/70	80	92	20	24
W F Duodenal ulcer	45	80	50	420	65	-22	3300	3450	108/80	106/80	88	100	16	20
F O Duodenal ulcer	55	70	65	380	65	17	2200	2300	108/76	108/85	102	100	20	20
S.S Arsphenamine hepatitis	50	50	140	450	110	15	3450	3550	104/60	106/76	70	74	12	16
Average	52	74	88	406	93	7	2730	2840	105/74	112/82	82	88	18	20

* P—prone
S—sitting
† Note the difference in the cisternal pressure in the two positions in each patient

TABLE 2

Congestive heart failure group

	Venous pressure		Spinal fluid pressure		Cisternal pressure †		Vital capacity		Arterial blood pressure		Pulse		Respirations	
	P*	S*	P	S	P	S	P	S	P	S	P	S	P	S
	mm H₂O	*mm H₂O*	*mm H₂O*	*mm H₂O*	*mm H₂O*	*mm H₂O*	*cc*	*cc*	*mm Hg*	*mm Hg*				
D H Syphilitic aortic insufficiency	215	230	320	415	310	50	900	1200	134/52	144/58	88	100	28	40
T W Hypertensive heart disease	310	355	400	680	355	100	1200	1400	158/128	152/116	60	86	40	44
H L Hypertensive heart disease	200	260	320	640	295	65	1000	1100	190/94	188/90	100	110	28	34
F W Hypertensive heart disease	310	345	360	480	365	80	1700	1900	160/100	154/98	80	88	20	22
L P Syphilitic aortic insufficiency	335	375	450	660	360	110	1100	1500	220/64	220/64	100	100	28	26
Average	274	313	374	575	337	81	1180	1420	172/87	171/85	85	96	28	33

* P—prone
S—sitting
† Note the marked difference in the cisternal pressure as well as the vital capacity in the two positions in each patient
This difference in the cisternal pressure in the two positions is much greater than in the control group

The venous pressure, the lumbar spinal fluid pressure, and the cisternal pressure in each position were found to be much greater in the patients with cardiac disease than in the normal individuals   The venous and the lumbar spinal fluid pressures in the patients with cardiac disease were also considerably higher in the sitting than in the prone position   As in the first group the cisternal pressure was much *less* in the upright than in the recumbent position   Although the difference in the cisternal pressure in the two positions in the normal individuals was considerable, in the patients with cardiac disease this difference was much greater and averaged 256 mm water   The arterial blood pressure, and pulse and respiration rates as in the first group showed no marked consistent changes between the two positions, although the pulse rate was usually slightly greater in the sitting position   In every case the vital capacity was higher in the sitting than in the prone position and the percentile increase in the vital capacity was greater in this group than in the patients without cardiac disease

COMMENT

Observations on the cisternal pressure of the normal individual in the sitting position have been rare   Ayer (6) reported the cisternal pressure in the upright position to be below zero   Weed (7) demonstrated the difference in cisternal pressure in animals in the two positions, and Weed and Hughson (8) have shown the close relationship that exists between the cerebrospinal fluid pressure and cerebral venous pressure It has been shown by Harrison, Cullen, Calhoun, Wilkins and Pilcher (9) that a decrease in vital capacity tends to cause a reflex increase in breathing   That an increase in venous pressure causes a reflex stimulation of respiration has been demonstrated by Harrison, Harrison, Calhoun, and Marsh (10)

Since the vital capacity is less in the prone than in the sitting position in patients with congestive heart failure, this decrease in vital capacity is no doubt one important cause of orthopnea   Furthermore, patients have been observed with slight orthopnea who have a diminution in vital capacity but no significant elevation in venous pressure or in cerebrospinal fluid pressure

On the other hand, a number of observations point to the conclusion that there is another very important factor not connected with the vital capacity concerned in the production of orthopnea   (1) In the first place, Ernstene and Blumgart observed no parallelism between the percentile reduction of vital capacity and the degree of orthopnea, but did observe a striking parallelism between the height of venous pressure and the degree of orthopnea   (2) Ernstene and Blumgart found that when patients with orthopnea were placed in the recumbent position, simple elevation of the head by flexing it on the thorax produced a

conspicuous diminution of respiratory distress, although this procedure had no significant effect on the vital capacity of the lungs   (3) Harrison found that, after spinal drainage was performed on individuals with orthopnea, many of these patients were able to breathe more comfortably in the prone position, although the vital capacity showed no significant change after this procedure   It has now been shown that the cisternal pressure is markedly decreased when a patient goes from the prone to the sitting position   It seems probable that this decrease in cisternal pressure along with its accompanying change in cerebral venous pressure explains the three sets of observations just mentioned   Anything that tends to decrease cisternal pressure in a patient with cardiac disease will give additional comfort in breathing   One method of reducing this pressure is to sit the patient up   Another method is to employ spinal drainage   Obviously, venesection is very useful, since it not only decreases venous and spinal fluid pressures, but also increases vital capacity

As a result of these observations it is believed that, in addition to the increase in vital capacity, the diminished cisternal pressure is an important factor in producing the relief from dyspnea obtained in the sitting position   The mechanism of this effect is as yet unknown

SUMMARY AND CONCLUSIONS

A study was made of the cisternal pressure, the lumbar spinal fluid pressure, the systemic venous pressure, the vital capacity, the arterial blood pressure, and the pulse and respiration rates in the recumbent and upright positions in five patients without cardiac disease and in five patients with congestive heart failure

1   The arterial blood pressure and pulse rate were usually slightly greater in the sitting than in the prone position, but in general the blood pressure, and the pulse and respiration rates showed no marked consistent changes with position in either group

2   The systemic venous and lumbar spinal fluid pressures were greater in the sitting than in the prone position in each subject, and were much greater in each position in the patients with congestive heart failure than in the patients without cardiac disease

3   The vital capacity was greater in the sitting than in the prone position in both groups of cases and the percentile increase of vital capacity with change in position was greater in the patients with cardiac disease

4   The cisternal pressure was much greater in each position in the group with congestive heart failure than in the other group

5   The cisternal pressure was much less in the upright than in the recumbent posture in each case and this difference in cisternal pressure between the two positions was much greater in patients with congestive heart failure than in those without cardiac disease

As a result of these observations it is believed that, in addition to the increase in vital capacity, the diminished cisternal pressure is an important factor in producing the relief from dyspnea obtained in the sitting position   The mechanism of this effect is as yet unknown

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THE ACTION OF PHLORIZIN ON THE EXCRETION OF GLUCOSE, XYLOSE, SUCROSE, CREATININE AND UREA BY MAN

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Although phlorizin has been used mostly in the lower animals, its administration to man is not without precedent, shortly after its isolation by de Koninck (1836a, b), this investigator tried it in the treatment of malaria on the ground that it was bitter, like other remedies which were effective in this disease The use of the drug in this connection was short-lived, however, and it was not until many years later that v Mering (1885) discovered that it caused glycuresis and diuresis, observations which initiated its application in the treatment of nephritis, sarcoma, etc , and as a test for renal function in man, in addition to its well known use in studies of metabolism Since these instances of human administration are of some interest, we have summarized them in Table I

The largest dose *per os* appears to be 15 grams in wafer form, reported by Piethiewicz (1869) and v Mering (1889), while Korte (1896) took three five gram doses dissolved in alcohol in one day v Mering (1889) also records the largest parenteral dose (in a case of sarcoma) 2 grams (in warm aqueous solution) injected subcutaneously daily, one gram in the morning and one gram in the evening, for a period of thirty days Benedict and Lewis (1913—personal communication) gave 2 grams daily in 10 cc of sterile olive oil subcutaneously over a period of days In no instance was permanent injury described

Moderate intravenous doses of the drug produce no unfavorable reactions in the dog, and we decided that the intravenous route would be the most satisfactory for our purposes, particularly since we wished to determine the exact effect of small doses Our anticipation in this matter has been justified, since we have given phlorizin in doses varying from 2 0 to 65 0 mgm per kgm with no unfavorable reactions other than an apparent diminution in glomerular activity with the larger doses We feel called upon, however, to express a word of caution about the use of the intravenous technique in man and refer the reader to the description of our method of preparation and administration Previous observations from this laboratory have shown that in the dog adequate doses of phlorizin raise the glucose clearance and lower the creatinine clearance to the level of the xylose clearance (Jolliffe, Shannon and Smith

TABLE I
Administration of phlorizin to man

Investigator	Date	Mgm per man per diem	Route of administration
de Koninck	1836	1,600	Per os, divided doses
v Coetsem	1836	1,600	Per os, one dose
Mareska	1836	1,600	Per os, 2 doses
de Muynck	1836	1,250	Per os, ?
Hanegraeff	1836	1,600	Per os, one dose
Pietkiewicz	1869	15,000	Per os, one dose
v Mering	1889	15,000	Per os, one dose
Klemperer	1896	10,000	Per os, one dose
Korte	1896	15,000	Per os, 3 doses
v Mering	1889	2,000	Hypodermic, 2 doses, for 30 days
Magnus-Levy	1896	1,000	Hypodermic, ?
Achard and Delamare	1899	50	Hypodermic, one dose
Delamare	1899	50	Hypodermic, one dose
Casper and Richter	1900	?	Hypodermic, one dose
Allard	1907	200	Hypodermic, one dose
Tanaka	1908	10	Hypodermic, one dose
Benedict and Lewis	1913	2,000	Hypodermic, one dose, repeatedly*
Chabanier and Lobo-Onell	1913	25	Hypodermic, one dose
Grote	1921	10	Hypodermic, one dose
Kamnitzer and Joseph	1921	2 5	Hypodermic, one dose
Hetényi	1922	60	Hypodermic, one dose
Rosenberg	1923	50	Intramuscular, one dose
Kamnitzer and Joseph	1924	2	Intramuscular, one dose
Dunner and Mecklenburg	1925	4 4	Hypodermic, one dose
Dunner and Kronenberger	1930	10	Intramuscular, one dose
Present experiments	1933	3,800	Intravenous, one dose

* In oil (personal communication)

(1932) and Shannon, Jolliffe and Smith (1932))   Our present experiments were designed to determine if similar changes in the glucose and creatinine clearances occur in man   Large doses of the drug were avoided, and different individuals were employed for each test

METHODS

Our subjects were convalescent patients, with normal renal function, kept in bed throughout the morning of the experiment   The routine of the experiments listed in Table II was as follows

The patient was allowed no breakfast   At zero minutes 70 grams of xylose were given in 10 tablespoons of oatmeal (no milk or sugar) and shortly afterward 10 grams of creatinine were taken in water   (N B   The administration of xylose in oatmeal circumvents the diarrhea which frequently follows its administration in aqueous solution and generally gives a flat blood sugar curve)   Varying amounts of water were given at zero, 30 and 60 minutes, and at 90 minutes the bladder was emptied (*vide infra*) and the first urine collection period begun   After one or more control periods phlorizin was injected

intravenously   During the next few minutes (washout period) normal saline was instilled into the bladder and removed with the discard   Three more specimens of urine were then collected at intervals of 20 to 30 minutes each

In the experiments listed in Table III the above routine was modified to permit the intravenous injection of sucrose   At zero minutes 500 cc of water were taken followed by 70 grams of xylose in 10 tablespoons of oatmeal, at 50 minutes an intravenous infusion of 10 per cent sterile sucrose solution (in distilled water) was begun, 600 cc being injected over a period of 30 to 50 minutes   Immediately after this injection, phlorizin was administered intravenously

Urine was collected by catheterization in dry flasks containing a little benzoic acid with special care to secure complete emptying of the bladder Samples of blood were taken from the antecubital vein as near to the middle of each urine collection period as possible   The blood was centrifuged and the plasma precipitated at once   All plasma concentrations were interpolated to the exact middle of the urine collection period and no correction was made for urinary dead space

Phlorizin, repurified by the method of Deuel and Chambers (1925) was weighed out in advance of the experiment   Just before use the phlorizin was dissolved in hot, freshly boiled 2 5 per cent $NaHCO_3$, and while still warm the solution was injected into the antecubital vein   In those experiments where the dose of phlorizin was greater than 2 5 grams, it was necessary to weigh the drug in two portions and to dissolve them separately   (Otherwise the solution cools and the drug recrystallizes during the injection)

The chemical methods used were those of Jolliffe Shannon and Smith (1932) and Shannon, Jolliffe and Smith (1932)   Sugar and creatinine analyses were done twice in all experiments except those on T S , M W , and M M the deviation between the two analyses was slight, and the recorded figures are in every case averages of the two determinations

Our experiments are divisible into two groups, in the first of which (Table II) the effects of single doses of phlorizin on the urea, glucose, xylose, and creatinine clearances were followed   Two control samples of blood and urine were collected prior to the injection of phlorizin, after a washout period of 20 to 50 minutes to clear the dead space of the kidneys, ureters and bladder, three more samples of urine and additional samples of blood were collected to enable us to observe the effects of phlorizin over a period of 40 to 60 minutes

The second group of experiments was designed to examine the effects of increasing doses of phlorizin on the glucose, xylose and sucrose clearances (Table III)   The arrangement of these experiments was similar to those of the first group except for the omission of control periods

In the first group of experiments 11 8 mgm of phlorizin per kgm raised the glucose clearance for at least one hour to the level of the xylose clearance, and 65 2 mgm per kgm did not raise the former significantly above the latter   In accordance with the observations previously published from this laboratory, we interpret these results to indicate that the last four individuals in Table II were "completely phlorizinized," i e , the tubular reabsorption of glucose was completely blocked

TABLE II

Simultaneous xylose, glucose, urea and creatinine clearances in man following intravenous administration of phlorizin

Subject	Phlo rizin	Sur- face area	Total con- current time	Urine flow per minute	$\frac{UV}{P}/SA$ Xy- lose	Glu- cose	Crea- tinine	Urea	$\frac{Creatinine}{Xylose}$	$\frac{Urea}{Xylose}$	$\frac{Glucose}{Xylose}$
	mgm per kilo	*sq m*	*minutes*	*cc*							
T S ♂	2 04	1 51	35	1 30	20 4		35 9	14 6	1 76	72	
			54	95	17 9		31 0	11 6	1 73	65	
			79	88	14 1		26 0	9 8	1 84	69	
			105	Washout period							
			141	2 17	22 6	13 0	33 2	15 0	1 47	66	58
			183	1 10	15 9	4 2	23 0	9 6	1 45	60	26
M W ♀	4 12	1 18	21	1 00	53 0		104 3	38 4	1 97	72	
			37	1 50	64 7		128 5	49 1	1 99	76	
			80	Washout period							
			99	4 60	55 0	35 2	106 0	46 1	1 96	84	64
			117	5 55	59 0	36 6	113 5	48 2	1 92	82	62
			138	5 76	59 1	30 8	122 4	47 1	2 07	80	52
M M ♀	6 07	1 52	31	3 20	18 4		43 2	14 5	2 35	79	
			63	3 20	34 3		78 5	26 5	2 29	77	
			90	Washout period							
			121	2 60	30 8	31 0	65 1	26 8	2 11	87	1 01
			150	3 60	37 6	34 6	86 4	32 5	2 30	86	92
			179	3 10	36 5	15 3	83 2	30 2	2 28	83	42
A H ♀	11 8	1 27	18	12 70	58 0		106 7	44 0	1 84	76	
			74	Washout period							
			94	3 20	49 1	50 3	86 2	38 8	1 76	79	1 02
			114	3 10	52 2	55 2	93 6	40 3	1 79	77	1 06
			139	2 90	55 4	57 6	94 0	40 8	1 70	74	1 04
M S ♀	15 7	1 39	25	1 60	53 5		96 3	41 7	1 80	78	
			72	Washout period							
			90	1 67	43 9	45 4	75 5	31 0	1 72	71	1 03
			104	1 63	42 3	44 2	73 0	30 7	1 73	73	1 04
			130	1 75	48 6	48 3	81 2	34 3	1 67	71	99
C S ♀	20 4	1 47	21	7 30	58 6		109 5	34 3	1 87	58	
			41	2 60	60 8		112 3	35 4	1 85	58	
			74	Washout period							
			94	2 37	52 4	54 0	105 5	30 6	2 01	58	1 03
			115	2 25	55 4	56 0	108 5	33 1	1 96	60	1 01
			139	2 26	58 0	56 8	110 9	34 9	1 91	60	98
N O ♀	65 2	1 60	20	9 70	43 0		76 3	33 1	1 77	77	
			44	6 62	41 4		82 5	34 3	1 99	83	
			91	Washout period							
			106	2 08	30 8	33 4	46 3	20 0	1 50	65	1 08
			126	2 10	32 9	34 3	49 9	20 7	1 52	63	1 04
			145	1 94	30 2	32 6	48 6	19 4	1 61	64	1 08

TABLE III

Simultaneous xylose sucrose and glucose clearances in man following intravenous administration of phlorizin

Subject	Phlorizin	Surface area	Total con current time	Urine flow per minute	$\dfrac{UV}{P}$/S.A.			$\dfrac{Glucose}{Xylose}$	$\dfrac{Sucrose}{Xylose}$
					Xylose	Glucose	Sucrose		
	mgm per kgm	*sq m*	*minutes*	*cc*					
A V ♀	21 7	1 58	18	6 64	57 1	45 8	56 0	0 80	0 98
			39	5 38	58 6	48 0	54 4	0 82	0 93
			60	4 41	50 6	43 2	48 5	0 85	0 96
			80	4 20	49 3	36 1	47 4	0 73	0 96
F M ♀	29 7	1 32	19	4 30	55 7	50 9	53 8	0 91	0 97
			39	3 95	55 4	55 4	53 6	1 00	97
			64	3 48	53 3	54 1	54 3	1 02	1 02
V T ♀	41 9	1 59	31	4 90	46 8	41 0	45 0	0 88	0 96
			51	3 80	37 8	34 7	35 6	92	94
			75	5 79	48 4	45 5	48 4	94	1 00
W M ♂	45 3	1 49	20	4 02	42 0	39 8	42 0	0 95	1 00
			43	3 97	47 0	45 9	46 1	0 98	98
			66	4 09	49 5	48 1	50 0	0 97	1 01
			87	3 77	47 8	48 1	47 4	1 01	99
F M ♀	59 4	1 32	29	4 30	55 1	56 0	58 6	1 02	1 06
			54	3 56	57 2	57 7	61 4	1 01	1 07
			82	2 86	46 2	44 8	48 5	97	1 05
			104	2 18	44 3	44 0	45 7	99	1 03

In the experiments listed in Table III, in which the comparison of xylose and sucrose was made, 21 7 mgm per kgm were apparently inadequate to produce complete glycuresis, but the variations in the glucose xylose ratio after larger doses must be considered to be within the experimental error   Again we pushed on to large doses to see if any one of the three sugars might be preferentially affected, but we find no evidence that such is the case   If xylose or sucrose were reabsorbed by the renal tubules, it might be imagined that a small dose of phlorizin would bring the glucose clearance up to the xylose clearance, while larger doses would raise the former definitely above the latter   To the contrary, it would seem that once the reabsorption of glucose is completely blocked, the simultaneous glucose, xylose and sucrose clearances remain identical within the experimental error, regardless of the quantity of phlorizin administered   In view of this fact, and in view of the further facts that the xylose clearance is not raised significantly in respect to the urea clearance, nor in respect to its own control level,[1] by the

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[1] The single exception to this statement occurred in T S, who received the smallest dose of phlorizin given

admmistration of phlorizin, we conclude that in man, as in the dog, the xylose (or sucrose) clearance is a measure of glomerular filtration *(cf* Jolliffe, Shannon and Smith, 1932)

Since, by the above interpretation, "complete phlorizination" consists of a change in renal activity whereby all the glucose filtered at the glomeruli is allowed to pass into the urine, and since the term "complete phlorizination" is open to several objections, we prefer to designate this condition as "complete glycuresis," in line with the use of this term as suggested by Benedict, Osterberg and Neuwirth (1918) to designate the increased rate of excretion of glucose, rather than the mere presence of this substance, in the urine   Thus, we are able to speak of complete glycuresis, transient glycuresis and recovery from glycuresis, a more flexible usage than is possible with the older term

The fact that the urea clearance is consistently lower than the xylose clearance is uninterpretable at the present time   The urea xylose ratios observed throughout this work confirm in general the observation of Jolliffe and Chasis (1933) and Keith, Power and Peterson (urea sucrose, 1933) on man, and our own observations on the dog

With regard to the effect of phlorizin on the creatinine clearance in man it will be noted that the administration of the drug resulted in a drop in the creatinine xylose ratio only in subjects T S and N O Since equally large variations in this ratio have been observed during the course of single experiments in man (unpublished details of Jolliffe and Chasis, 1933), we cannot attach particular significance to this result, and we conclude that larger doses of phlorizin are required to reduce the creatinine clearance to the level of the xylose clearance (i e to depress completely the tubular secretion of creatinine) than are required to produce complete glycuresis   It would be of interest to know if complete depression of creatinine secretion could be obtained in man as in the dog, but we decided that the question did not justify the administration of intravenous doses larger than those we have given

In conclusion, it may be noted that the glycuresis induced in man by phlorizin is transient, as is the case in the dog   All our subjects were tested for glycosuria at intervals following the formal observations, and found to be essentially sugar-free within 24 hours

SUMMARY

The minimum intravenous dose of phlorizin required in man to produce complete phlorizination (i e , to raise the glucose clearance to xylose clearance) was found to be in the neighborhood of 10 to 20 mgm per kgm

Sixty-five mgm per kgm did not raise the glucose clearance above the xylose clearance, and 59 4 mgm per kgm did not cause deviation between the xylose and sucrose clearances   Neither did the phlorizin

cause a significant rise in the xylose clearance as compared to control periods taken just before the administration of the drug

In the largest dose which we have given (65 0 mgm per kgm) the drug exerted no significant depressive action on the ratio of creatinine xylose clearance

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AMINO NITROGEN CHANGES OF THE BLOOD IN NEPHRITIS

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Amino nitrogen and peptide nitrogen content of the blood in nephritic patients have been frequently determined since the introduction of suitable micro methods. Gasometric determinations of amino nitrogen and peptide nitrogen of plasma and red blood cells separately have, however, not yet been done

That colorimetric analyses of the same blood samples give lower results for amino acid nitrogen than the gasometric method or the formaldehyde titration, and that colorimetric recovery of amino acids added to blood is incomplete, have been reported by Van Slyke and Kirk (18) The use of different analytical methods may accordingly explain the conflicting results reported in the literature. Thus most observers using the colorimetric method of Folin (Berglund (2), Greene, Sandiford and Ross (6), Schmidt (16), Witts (20), Feinblatt and Shapiro (5)) found no deviation from the normal in series of patients with nephritis, including several cases of uremia. Slightly or moderately increased values of amino nitrogen in uremia were reported with the colorimetric method by Wowsi and Gelbird (21), and by Looney (11) in an individual case of mercuric chloride poisoning shortly before death. Extensive studies have been made in recent years in Volhard's clinic by Becher and Herrmann (1) with a modification of Folin's colorimetric technique, increase of free amino nitrogen of whole blood was frequently observed in patients with severe impairment of the kidney function. Only in a single case, however, did the method used indicate values exceeding 10 milligrams per cent

Most of the work done with the gasometric method is of earlier date. Using various protein precipitants Bock (3), Okada and Hayashi (14), and Desqueyroux (4), all found increased amino nitrogen of the blood in cases of advanced nephritis. In nephritic toxemias of pregnancy, on the other hand, Losee and Van Slyke (12), and Morse (13) observed no marked deviation from the normal

Quantitative determination of peptide nitrogen of blood was first made with exact methods by Hiller and Van Slyke in 1922 (7) by gasometric determination of blood filtrates before and after hydrolysis with hydrochloric acid. A similar procedure of acid hydrolysis has been used by different authors in studies of the peptide nitrogen in nephritis,

but in most cases other methods were substituted for the gasometric to determine the amino nitrogen before and after hydrolysis   When comparing results both the method of amino nitrogen determination and the protein precipitant used should be considered   Hulse and Strauss (9), using the formaldehyde titration method of Sørensen, and Hulse and Franke (8), using the gasometric method, observed high values for peptide nitrogen in nephritic patients with hypertension   Jackson, Sherwood and Moore (10), Becher and Herrmann (1), and Schlossmann (15), using the colorimetric method, were, however, unable to find any relation between the blood pressure and the content of peptide nitrogen in the blood in nephritis   A definite increase in peptide nitrogen was frequently seen by Becher and Herrmann (1) in cases of severe renal insufficiency, the peptide nitrogen even occasionally exceeding the concentration of free amino nitrogen, increase of peptide nitrogen with normal amino nitrogen values was sometimes observed

METHODS

For separate analyses of cells and plasma, oxalated blood was centrifuged till constant volume of the red blood cells was obtained, plasma was then syphoned off and the intermediate layer of plasma and cells discarded Samples of red blood cells were measured with a calibrated pipette "to contain "

Urea was transformed into ammonia by addition of a urease solution and phosphate buffer as described by Van Slyke (17)   The proteins of plasma or whole blood were then precipitated by the Folin-Wu method   To plasma or whole blood one volume of 10 per cent sodium tungstate and one volume of 2/3 N sulfuric acid were added, and the mixture was diluted with water to 10 times the original volume of the sample   For precipitation of the proteins of the red blood corpuscles two volumes of 10 per cent sodium tungstate and two volumes of 2/3 N sulfuric acid were used for each volume of cells, but the sample was finally, as in precipitation of plasma and whole blood, made up to 10 times its original volume with water   Five cc of filtrate, therefore, represented 0 5 cc of plasma, red blood corpuscles or whole blood   Usually, however, the filtrate from the red blood corpuscles was again diluted with two volumes of distilled water to obtain sufficient material for analysis   Because of the high amino nitrogen content of the red blood cells the dilution did not diminish the amino nitrogen readings enough to interfere with accuracy

The ammonia of the filtrates, formed by the splitting of the urea with urease, was removed by boiling with milk of magnesia and amino nitrogen afterwards determined by the manometric method of Van Slyke (17)

For determination of peptide nitrogen the urea-and-ammonia-free filtrates were heated on a water bath for 24 hours with equal volumes of concentrated hydrochloric acid   After evaporation of the acid in an open dish each residue was neutralized with a few drops of a saturated solution of sodium acetate and finally made up to volume (7)   It was found unnecessary to subject the hydrolyzed samples again to boiling with milk of magnesia before the amino acid determination, as identical results were obtained with and without such treatment

For blank analyses a urease solution was prepared, and precipitated, and

the filtrate was diluted as described by Van Slyke (17 p 442), or Peters and Van Slyke (22)   The amino nitrogen content of the filtrate was determined before and after hydrolysis, to obtain the c correction   Separate blanks were determined for analyses of plasma and red blood corpuscles

The normal magnitude and constancy of results obtained with the above procedure is illustrated by Table I   It gives the results from two samples of the same blood which were centrifuged and analysed separately   The sample of plasma filtrate represented 0 5 cc of plasma, the sample of filtrate of red blood cells 0 167 cc of corpuscles

TABLE I

Showing reproducibility of measurements

	Milligrams per cent amino nitrogen	
	I	II
Plasma unhydrolyzed	4 10	4 07
Plasma hydrolyzed	4 10	4 03
R B C unhydrolyzed	12 85	12 91
R B C, hydrolyzed	18 89	18 03

Studies of amino nitrogen and peptide nitrogen of the blood were made in seven uremic patients   (See Tables II and III)

TABLE II

Amino nitrogen in whole blood during development of uremic coma
Case No 1, M J   Hospital No 7855

Date	Amino nitrogen per 100 cc.			Whole blood nonprotein nitrogen per 100 cc.	Whole blood urea nitrogen per 100 cc.	Urea clearance	Condition
	Plasma	Cells	Whole blood				
1931	mgm	mgm	mgm	mgm	mgm	*per cent of mean normal*	
September 27			18 0	148	118		Uremia
September 28			13 7	150	119	6	Uremia
October 13							
10 00 A M }			12 3	171	191		Uremia }
9 30 P M ∫			21 4	189	141		Coma ∫
October 29	28 8	36 7					Coma

CLINICAL NOTES ON UREMIC CASES [1]

Case No 1   Hospital No 7855   M J , female, 33 years   As a child had

[1] The nomenclature followed here for the different types and stages of Bright s disease is in general that used by Van Slyke Stillman et al (19) In addition, to distinguish the different conditions near and in coma, the following terms are used
Terminal stage with nitrogen retention   Nitrogen retention without clinical symptoms
Uremia   Nitrogen retention with clinical symptoms, such as nausea and vomiting but with normal consciousness
Semi-comatose condition   Consciousness greatly influenced, but patient still responds
Coma   Patient unconscious and does not respond

TABLE III

Uremic cases 2, 3, 4, 5 and 6

Subject			Amino nitrogen per 100 cc				Blood urea nitrogen per 100 cc	Urea clearance	Condition
			Plasma		Cells				
Initials and serial number	Hospital number	Date	Free	Peptide	Free	Peptide			
			mgm	*mgm*	*mgm*	*mgm*	*mgm*	*per cent of mean normal*	
E H No 2	8166	1932							
		May 29	23 4	−1 0	12 2	16 5			Uremia
		June 2	37 3	7 0	24 8	18 0		2	Coma
J D No 3	8080	1932							
		June 29	16 8	−0 6	12 4	6 3			Uremia
		July 7	12 3	1 0	18 4	17 0			Uremia
E B No 4	7884	1931							
		November 5	5 9	1 9	13 5	12 0		61	Good
		November 6	6 9	−0 2	15 3	2 9		90	
		December 21	6 5	0 4	13 1				
		1932							
		May 26	6 5		14 1				
		June 1	6 6		12 4				
		August 29							
		September 2	21 3	−5 1	12 9	9 5	140	8	Coma Died 48 hours later

TABLE III (continued)

Subject		Date	Amino nitrogen per 100 cc.				Blood urea nitrogen per 100 cc.	Urea clearance	Condition
Initials and serial number	Hospital number		Plasma		Cells				
			Free	Peptide	Free	Peptide			
			mgm.	*mgm*	*mgm*	*mgm*	*mgm*	*per cent of mean normal*	
M W No 5	6473	**1931**							
		December 6	21 80	-2 29	19 24	10 56	163		Coma Intravenous glucose
		December 10	22 40	-1 99	16 25	9 85	143	7 6	Still comatose but condition improved
		December 15	10 24	92	11 80	8 26			Condition improved
		December 16	7 18	1 33	12 90	8 25			Condition improved
		December 17	5 82	1 66	13 94	7 00			Condition good
		December 21	21 12	12	14 32	8 08	151		Ascites developing
		December 22	7 29	1 27	14 70	7 28	152		Fever (till December 24)
		December 26	10 20	1 20	10 67	7 32	157		Condition good
		December 29	8 19				127	7 2	Condition good
		1932							
		January 1	5 95	10 21	16 54	7 19	126		Condition good
		January 5	6 93	33	14 45	13 54	118		Condition good
		January 11	11 52	17	16 07	9 36	91	8 4	Condition good
		May 1	14 22	71	15 51	9 27	95	7 1	Condition good
		September 21						3 4	
		September 26	28 58	2 42	7 10	9 38	239		Coma
		September 27	28 20	-1 58	9 80	2 81	295		Coma

TABLE III (continued)

Subject		Date	Amino nitrogen per 100 cc				Blood urea nitrogen per 100 cc	Urea clearance	Condition
Initials and serial number	Hospital number		Plasma		Cells				
			Free	Peptide	Free	Peptide			
			mgm	*mgm*	*mgm*	*mgm*	*mgm*	*per cent of mean normal*	
M R No 6	7872	1931							
		November 24	5 57	− 18	12 24	9 28	20	26	Good
		December 3	11 63	−1 62	13 80	5 05	33	22	Good
		1933							
		May 21	6 43				82	7	Good
		June 13	9 06				182		Uremia
		June 14							
		9 30 A M	13 76						Semicomatose Sample taken before i v glucose injection
		9 30 P M	10 87						Semicomatose Sample taken before 2nd i v glucose injection
		11 00 P M	14 72						Semicomatose
		June 15							
		6 45 A M	10 20						Semicomatose
		11 45 A M	15 46						Moment of death
M C No 7	8392	See Figure 1							

hemorrhagic Bright's disease following tonsillitis   After several years of active symptoms the patient went into a latent stage, but the renal lesion became active again during a pregnancy, necessitating abortion   In the following period of ten years exhibited hypertension and proteinuria   Two weeks before admission vomiting, edema of feet and dyspnea developed   On admission (September 25 1931) was in uremia

   Case No 2   Hospital No 8166   E H, female, 21 years   Two years before admission had hematuria and hypertension   Visual disturbances, edema of ankles, vomiting and diarrhea had been present in the last weeks   On admission (May 28, 1932) was in uremia

   Case No 3   Hospital No 8080   J D, male, 30 years   Ten months before admission developed tonsillitis proteinuria and hypertension, followed by edema and ascites   In the last days had had severe nose bleedings   On admission (June 28, 1932) was in uremia

   Case No 4   Hospital No 7884   E B, male 29 years   Nine years before admission had had respiratory infection with hematuria   Recovered into a latent stage with proteinuria as only symptom   In the last six months had had hypertension and severe headache   The patient was observed for several months during the chronic active stage   and during the last weeks of terminal uremia

   Case No 5   Hospital No 6473   M W, male, 15 years   Three and a half years before admission developed generalized edema with proteinuria and reduced kidney function following an attack of upper respiratory infection   In the following period gave evidence of activity in the kidney lesion (microscopic hematuria)   Immediately before admission (December 3, 1931) suffered from abnormal sleepiness and attacks of convulsions

   Case No 6   Hospital No 7872   M R, female, 19 years   Four months before first admission had edema of the legs   On admission (October 21, 1931) macroscopic hematuria   hypertension and reduced kidney function were present   In the following 18 months the nephritis progressed through the chronic active to the terminal stage   Two weeks before last admission (May 4, 1933) reduction of vision and occasional cardiac decompensation were noted   Death occurred on June 15 1933, after two days of a semicomatose condition

   Case No 7   Hospital No 8342   M C   male 19 years   Four years before admission developed proteinuria (and hematuria ?) following mastoiditis   Went into a latent stage of hemorrhagic Bright s disease   Three weeks before admission had had inflammation of the mandibular region caused by hemolytic streptococci   In relation to this infection there occurred acute exacerbation of the nephritis with oliguria, gross nitrogen retention and vomiting   On admission (November 25, 1932) was in acute uremia, markedly dehydrated   During the first days in the hospital was at intervals extremely drowsy, but not comatose

   Following treatment of the dehydration the patient made a quick recovery to an excellent general condition   The activity of the renal lesion persisted however, and the kidney function never exceeded 10 per cent of the average normal urea clearance
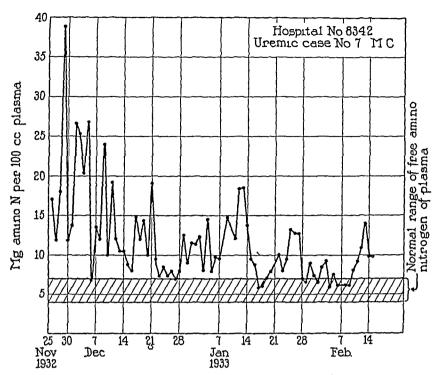
   Amino nitrogen determinations of plasma were made daily for a period of about three months   The data are presented in Figure 1   The highest value, 38 7 mgm of amino nitrogen per 100 cc of plasma, was observed on November 29, 1932 at a time when the patient was very drowsy

TABLE IV

Amino nitrogen and peptide nitrogen content of blood of normal individuals and non-uremic nephritic patients

Subject	Date	Amino nitrogen in plasma		Amino nitrogen in red blood cells		Urea clearance	Diagnosis
		Free	Peptide	Free	Peptide		
		mgm per 100 cc	*mgm per 100 cc*	*mgm per 100 cc*	*mgm per 100 cc*	*per cent of mean normal*	
A A	September 27, 1932	5 20	− 54	11 10	6 95		Normal
S S	August 7, 1932	6 56	55	11 09	6 37		Normal
E K	December 10, 1931	5 29	41	18 07	5 01		Normal
Same	January 21, 1932	4 48	16	13 14	7 27		
Same	April 15, 1932	5 51	17	20 51	9 32		
M S	September 28, 1932	4 33	− 06	7 99	5 12		Normal
J M	September 28, 1932	4 93	− 30	10 15	4 85		Normal
W M	September 29, 1932	4 14	19	8 26	3 95		Normal
Hospital Number							
7943	January 4, 1932	5 46	23	12 44	7 01	78	Hemorrhagic Bright's disease initial
7986	April 7, 1932	5 93	37	14 54	4 12	30	Same
7828	November 25, 1931	5 12	72	10 21	5 98	109	Same
7872	November 24, 1931	5 57	− 18	12 24	9 28	26	Same
Same	December 3, 1931	11 63	−1 62	13 80	5 05	22	Same
7450	November 19, 1931	4 45	− 15	10 98	6 82	92	Hemorrhagic Bright's disease active
7842	January 6, 1932	4 90	− 32	8 33	6 53	44	Same
7884	November 5, 1931	5 87	1 86	13 46	11 97	60	Same
Same	November 6, 1931	6 92	− 22	15 29		60	Same
Same	December 21, 1931	6 48	44	13 09	2 89	90	Same
7938	December 17, 1931	6 49	19	8 89	9 57	88	Hemorrhagic Bright's disease latent
7874	November 19, 1931	5 08	− 15	13 60	5 64	161	

TABLE IV (continued)

Subject	Date	Amino nitrogen in plasma		Amino nitrogen in red blood cells		Urea clearance	Diagnosis
		Free	Peptide	Free	Peptide		
		mgm per 100 cc	mgm per 100 cc.	mgm per 100 cc	mgm per 100 cc.	per cent of mean normal	
7923	December 2 1931	7 83	1 01	13 55	5 55	17	Hemorrhagic Bright's disease terminal
Same	December 10 1931	7 84	- 23	17 74	6 86	15	Same
Same	April 14, 1932	10 45	33	19 69	10 55	10	Same
7867	October 24 1931	7 83		15 85		19	
8101	April 23, 1932	5 00	47	16 40	6 47	18	Myeloma Bence-Jones proteinuria
7922	January 3, 1932	8 21	-1 47	12 56	5 79	36	Degenerative Bright's disease same (Etiology pregnancy)
7905	November 13 1931	4 90	26	13 56	6 07	97	
Same	November 30 1931	4 05	16	12 11		90	Same
7963	January 25 1932	5 58		16 07		14	Arteriosclerotic Bright's disease
7837	December 29 1931	4 08	01	12 88	5 58	54	Same
8063	April 8 1932	4 71	1 43	13 94	1 94	35	Same
7876	November 9 1932	7 78	54	13 69	5 81	61	Hemorrhagic Bright's disease with nephrotic component
Same	November 10 1931	6 88		13 55	7 46	61	Same
Same	November 24 1931	8 04	- 57	12 73	8 50	57	Same
Same	December 3, 1931	5 66	-1 66	10 25	6 07	58	Same

FIG 1   AMINO NITROGEN CONTENT OF BLOOD PLASMA DURING AND AFTER
RECOVERY FROM ACUTE UREMIA

SUMMARY OF RESULTS

In cases of chronic and acute nephritis with more than 40 per cent
of normal urea clearance the plasma amino nitrogen content, determined
gasometrically, was found normal, 4 to 6 5 mgm per 100 cc

In more advanced cases, but without uremic symptoms, the plasma
amino nitrogen was sometimes normal and sometimes increased to 8 to
10 mgm per 100 cc

In the pre-coma stage, the plasma amino nitrogen was variable, and
might change in 24 hours from practically normal to over 20 mgm per
100 cc

In uremic coma, the plasma amino nitrogen was found uniformly
high, from 2 to 7-fold normal

The amino nitrogen fluctuations in the plasma were more marked
than in the cells, and more regularly related to the condition of the
patients   Significant variations in the plasma peptide nitrogen were not
observed

CONCLUSIONS

Elevation of plasma amino nitrogen tends to be more frequent as
renal disease becomes more advanced   The correlation between fall in

renal function and rise in plasma amino nitrogen is irregular, however, in some cases the renal clearance approaches the coma level (about 5 per cent of normal clearance) before rise in plasma amino nitrogen occurs It appears probable, therefore, that the rise in amino acid content is not directly due to renal failure, but to a breakdown in the mechanism for metabolism of the amino acids occurring elsewhere as part of the general debacle

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A FORMULA AND NOMOGRAM FOR THE ESTIMATION OF THE OSMOTIC PRESSURE OF COLLOIDS FROM THE ALBUMIN AND TOTAL PROTEIN CONCENTRATIONS OF HUMAN BLOOD SERA

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In connection with investigations to be reported subsequently the osmotic pressure of colloids and the albumin and total protein concentrations of 128 samples of human blood serum were determined From these data, which cover a wide range of values, an empirical formula has been evolved which, it is believed, will enable the osmotic pressure to be more accurately estimated from the protein analyses of human sera than is the case with formulae now employed for this purpose (1, 2) A nomogram has been constructed to facilitate such computations The simple, definitely linear relation found to exist between the albumin concentration and the specific osmotic pressure would indicate that the albumin globulin ratio is not as reliable an index of the osmotic prop erties of serum as is at present often assumed

It may justly be contended—at least on theoretical grounds—that the accuracy of most of the colloid osmotic pressure measurements previously carried out on human blood sera is open to serious question, either for the reason that the authors have failed to recognize the fundamental criteria of equilibrium upon which any good physical chemist would insist (3) or because of the failure to describe the methods employed in sufficient detail to enable one to judge as to whether such criteria have actually been fulfilled

In a recent communication (4) it was emphasized that, for the accurate determination of the osmotic pressure of colloids, it is essential to observe certain rigid criteria of an osmotic equilibrium Procedures were described by which these criteria of accuracy might be fulfilled, and a detailed description of the apparatus and methods has recently been published (5) By a strict adherence to such standards it has been possible, during the present investigation, to obtain from duplicate determinations on each serum pressure values which, in 95 per cent of the cases, do not differ by more than 12 mm of water No pressure reading has been considered to be accurate unless it has been possible to maintain the volume of the serum at a constant value, at this pressure

and at constant temperature, for a period of at least two hours—a period of observation sufficient, according to our tests, to insure the attainment of a definite equilibrium when the membranes are of the standard selected permeability   Consequently, it is believed that our figures actually refer to equilibrium pressures   The fact that the standard per cent deviation of the calculated from the observed values of the osmotic pressure is only ± 5 per cent leads us to believe, furthermore, that for routine clinical purposes, the method of calculation, which avoids the many difficulties inherent in the usual methods of direct determination, will provide a sufficiently reliable index of the water-retaining power of the serum   Such a conclusion is, however, to be considered as subject to the same qualifications as apply to all cases of indirect measurements, as for example, the estimation of the basal metabolic rate from the oxygen consumption   The possibilities of error in the use of the calculated figures will be discussed in later paragraphs

METHODS

Protein analyses were carried out in duplicate by the macro-Kjeldahl method on samples drawn under oil without stasis   Globulin was separated by precipitation at 38° C with 22 5 per cent sodium sulphate according to the method of Howe (6)   Nonprotein nitrogen was estimated by the micro-Kjeldahl-Nesslerization method, on oxalated plasma The usual factor, 6 25, was used to convert protein nitrogen values to protein concentrations

The osmotic pressure determinations were carried out in a constant temperature room at 20° to 22° C   Duplicate determinations were done in all but four instances   The collodion membranes used were standardized to a specific permeability (4) range of 20 × 10⁻⁸ to 40 × 10⁻⁸ Equilibrium was usually attained in 1 to 3 hours, but a somewhat longer time was occasionally required   The details of the method and the criteria of osmotic equilibrium have been described elsewhere (4, 5)   Occasional lapses of the vigilant attention to detail which is necessary for success in carrying out such determinations invariably led to gross errors which necessitated discarding the results   On the other hand, all data have been included for which definite evidence of gross errors of technic was lacking, regardless of the degree of variation between the duplicate determinations

DATA

The blood samples were taken from 53 individuals, from 1 to 10 determinations being carried out on each person, often over periods of several months   Of these 53 persons 14 were laboratory workers, students, and other volunteers, all apparently in good health, 20 presented, at one time or another, and to various degrees, evidences of nutritional edema, 12 had heart disease, and 1 each suffered from the following

pathological conditions multiple xanthomatosis, chronic diarrhea, aplastic anemia, pellagra with chronic colitis, Banti's disease, nephrosis, syphilitic cirrhosis of the liver

The data are presented in Table I, arranged in order of increasing albumin concentrations   The 10 lowest albumin, total protein and osmotic pressure figures were obtained from a single individual, over a period of 5 months during the gradual decline associated with an advanced nephrosis   The 13 highest values were obtained from blood which had been concentrated by causing the subjects to stand in the erect posture for one hour or longer, in connection with experiments which are to be described elsewhere   The extreme range of the values encountered may be indicated by the following figures   albumin, per cent, 0 89 to 6 62, globulin, 1 66 to 6 48, total protein, 2 99 to 9 64, albumin globulin ratio, 0 42 to 2 80, osmotic pressure of colloids, 76 to 583 mm water, and specific osmotic pressure, 25 4 to 59 2 mm water   The figures for the albumin concentrations probably furnish the best index as to the distribution of the values   There were 10 albumin values below 2 5, 13 between 2 5 and 3 5, 50 between 3 51 and 4 5, 48 between 4 51 and 5 5, and 7 above 5 5

ANALYSIS OF THE DATA

The specific osmotic pressure, S, was calculated for each case by dividing the value of the total osmotic pressure, P, measured in mm of water, by the concentration of total proteins, C, measured in grams per 100 cc   Values for the specific pressures were plotted on a three-dimensional graph against the corresponding values of the albumin and of the globulin concentrations   This was done by driving wires, sharpened at one end, into the drawing board on which the axes of albumin and globulin concentrations (A and G) were laid off on cross section paper   Each value of S was thus represented by the height of a wire and the point through which the wire was driven represented the corresponding A and G values   The surface produced by the tips of the wires was seen to be fairly regular, but contrary to our earlier expectations it did not show any obvious relation between the specific osmotic pressure and the globulin concentration or A/G ratio   The formula of Govaerts (1) implies the assumption that the specific pressure is determined primarily by the A/G ratio   According to his concept the value of S will fall with increasing values of G for sera having a constant value of A Govaerts also assumes that the total protein concentration, C, is without effect on the value of S in the case of sera which have the same A/G ratio   In our series there is an obvious and regular relation between C and S and it was apparent from inspection of the wire model, that the increasing values of S, as C increased, were due entirely to the increase in A The relation between the specific pressure and the albumin was

TABLE I

Data arranged in ascending order of albumin concentrations

Case number	Albumin	Specific pressure observed	Specific pressure calculated	Total protein	Osmotic pressure observed	Osmotic pressure calculated	Variation of calculated from observed pressure	Maximum experimental error	Unexplained variations
	grams per 100 cc	mm H_2O	mm H_2O	grams per 100 cc	mm H_2O	mm H_2O	mm H_2O	mm H_2O	mm H_2O
37	0 89	25 4	26 7	2 99	76	80	+ 4	±21	
37	1 25	30 9	28 8	3 10	96	89	- 7	±22	
37	1 46	29 3	30 0	3 35	98	101	+ 3	±22	
37	1 75	30 1	31 7	3 56	107	113	+ 6	±23	
37	1 93	31 2	32 8	3 59	112	118	+ 6	±23	
37	1 93	30 4	32 8	3 71	113	122	+ 9	±23	
37	1 94	31 3	32 8	3 89	122	128	+ 6	±23	
37	2 28	38 6	34 9	4 09	158	143	-15	±24	
37	2 41	34 6	35 6	4 16	144	148	+ 4	±24	
37	2 44	32 2	35 8	4 28	138	153	+15	±24	
35	2 57	34 1	36 6	4 36	149	159	+10	±25	
26	2 67	39 5	37 2	5 92	234	220	-14	±27	
20	2 80	43 6	37 9	5 92	258	224	-34	±27	- 7
36	2 87	41 5	38 3	9 35	388	358	-30	±31	
49	3 11	40 9	39 7	4 88	199	194	- 5	±26	
47	3 19	39 7	40 2	5 74	228	231	+ 3	±27	
47	3 19	41 0	40 2	5 68	233	228	- 5	±27	
47	3 20	39 0	40 3	5 84	228	235	+ 7	±27	
49	3 30	43 4	40 9	4 98	216	204	-12	±26	
49	3 30	39 6	40 9	5 10	202	208	+ 6	±26	
47	3 34	40 8	41 1	5 64	230	232	+ 2	±27	
49	3 46	39 8	41 8	5 18	206	217	+11	±27	
32	3 49	42 7	42 0	7 61	325	320	- 5	±30	
49	3 52	47 6	42 2	5 15	245	217	-28	±27	- 1
31	3 60	43 4	42 6	6 38	277	272	- 5	±28	
49	3 61	45 7	42 7	6 10	279	260	-19	±28	
31	3 62	43 7	42 8	6 58	288	281	- 7	±28	
47	3 67	36 3	43 1	6 71	244	289	+45	±29	+16
31	3 67	37 5	43 1	6 33	237	273	+36	±28	+ 8
23	3 70	41 3	43 2	6 27	259	271	+12	±28	
49	3 70	42 1	43 2	5 25	221	227	+ 6	±27	
38	3 73	44 6	43 4	7 15	319	310	- 9	±29	
32	3 73	46 3	43 4	8 09	375	351	-24	±31	
32	3 80	42 6	43 8	7 44	317	326	+ 9	±30	
52	3 82	43 0	43 9	6 45	277	283	+ 6	±29	
23	3 83	46 4	44 0	6 66	309	293	-16	±29	
33	3 94	43 3	44 6	6 50	281	290	+ 9	±29	
42	3 98	45 9	44 9	6 99	321	314	- 7	±29	
48	4 01	44 9	45 1	6 82	306	307	+ 1	±29	
22	4 03	45 0	45 2	6 71	302	303	+ 1	±29	
38	4 04	43 8	45 2	6 98	306	316	+10	±30	
23	4 04	45 7	45 2	7 12	325	322	- 3	±30	
10	4 05	47 6	45 3	6 49	309	294	-15	±29	
10	4 10	49 1	45 6	6 38	313	291	-22	±29	

TABLE I (continued)

Case number	Albumin	Specific pressure observed	Specific pressure calcu lated	Total protein	Osmotic pressure observed	Osmotic pressure calcu lated	Varia tion of calcu lated from observed pressure	Maxi mum ex perimen tal error	Unex plained vari ations
	grams per 100 cc.	mm H₂O	mm H₂O	grams per 100 cc	mm H₂O	mm H₂O	mm. H₂O	mm H₂O	mm H₂O
7	4 11	44 2	45 6	7 95	352	363	+11	±31	
12	4 13	47 1	45 8	6 33	298	290	− 8	±29	
16	4 14	44 1	45 8	7 01	309	321	+12	±30	
33	4 16	44 3	45 9	6 77	300	311	+11	±29	
23	4 19	44 2	46 1	6 92	306	319	+13	±30	
4	4 22	46 4	46 3	7 06	328	327	− 1	±30	
31	4 22	49 2	46 3	7 54	371	349	−22	±30	
17	4 23	45 8	46 4	7 08	324	328	+ 4	±30	
17	4 25	49 4	46 5	6 82	337	317	−20	±30	
17	4 27	47 7	46 6	6 67	318	311	− 7	±29	
28	4 27	48 6	46 6	5 99	291	279	−12	±29	
24	4 30	49 1	46 8	6 49	319	304	−15	±29	
3	4 33	47 2	46 9	6 65	314	312	− 2	±29	
50	4.33	45 5	46 9	8 18	372	384	+12	±31	
3	4 36	48 7	47 1	6 47	315	305	−10	±29	
29	4 37	48 0	47 2	7 06	339	333	− 6	±30	
6	4 38	45 4	47 2	7 11	323	336	+13	±30	
25	4 39	45 0	47.3	7 25	326	343	+17	±30	
4	4 39	48 0	47 3	7 11	341	336	− 5	±30	
17	4 40	47 7	47 4	6 64	316	314	− 2	±30	
9	4 41	47 3	47 4	7 06	334	335	+ 1	±30	
16	4 42	46 4	47 5	6 70	311	318	+ 7	±30	
8	4 42	47 2	47 5	6 80	321	323	+ 2	±30	
2	4 43	42 8	47 5	6 48	277	308	+31	±29	+ 2
30	4 44	47 0	47 6	7 10	334	338	+ 4	±29	
15	4 46	49 8	47 7	6 26	312	299	−13	±29	
44	4 46	48 4	47 7	6 92	335	330	− 5	±30	
43	4 48	51 2	47 8	6 64	340	318	−22	±30	
9	4 49	48 9	47 9	6 99	342	335	− 7	±30	
29	4 50	45 7	48 0	7 10	325	340	+15	±30	
8	4.50	51 0	48 0	7 12	363	341	−22	±30	
24	4 52	46 3	48 1	6 78	314	326	+12	±30	
29	4 52	44 9	48 1	7 07	317	340	+23	±30	
5	4 52	49 8	48 1	6,33	315	304	−11	±29	
17	4 53	47 2	48 1	7 44	351	358	+ 7	±31	
3	4 54	48 3	48 2	6 63	320	319	− 1	±30	
51	4 55	52 8	48 2	6 35	335	306	−29	±29	
21	4 57	49 2	48 4	6 69	329	324	− 5	±30	
8	4 59	46 8	48 5	6 72	314	326	+12	±30	
30	4 59	49 6	48 5	6 86	340	333	− 7	±30	
30	4 60	48 8	48.5	7 27	355	353	− 2	±31	
30	4 62	44 8	48 7	7 12	319	346	+27	±30	
39	4 62	48 6	48 7	6 85	333	333	0	±30	
8	4 62	48 2	48 7	6 91	333	336	+ 3	±30	
15	4 63	51 2	48 7	6 53	334	318	−16	±30	
41	4 64	51 5	48 8	6 87	354	335	−19	±30	

TABLE I (*continued*)

Case number	Albumin	Specific pressure observed	Specific pressure calculated	Total protein	Osmotic pressure observed	Osmotic pressure calculated	Variation of calculated from observed pressure	Maximum experimental error	Unexplained variations
	grams per 100 cc	*mm H₂O*	*mm H₂O*	*grams per 100 cc*	*mm H₂O*	*mm H₂O*	*mm H₂O*	*mm H₂O*	*mm H₂O*
6	4 68	47 6	49 0	6 74	321	330	+ 9	±30	
24	4 68	51 6	49 0	7 02	362	344	−18	±30	
18	4 69	50 5	49 1	7 92	400	389	−11	±31	
24	4 72	48 9	49 2	7 12	348	351	+ 3	±31	
34	4 72	50 5	49 2	6 87	347	338	− 9	±30	
46	4 77	50 3	49 5	7 51	378	372	− 6	±31	
11	4 77	50 0	49 5	7 13	357	353	− 4	±31	
24	4 78	53 3	49 6	6 64	354	329	−25	±30	
13	4 78	50 4	49 6	6 91	348	343	− 5	±30	
19	4 78	49 1	49 6	7 03	345	349	+ 4	±31	
53	4 79	51 9	49 7	7 14	371	355	−16	±31	
27	4 81	51 4	49 8	7 02	361	349	−12	±31	
12	4 84	51 4	50 0	7 50	385	375	−10	±31	
15	4 90	49 3	50 3	6 78	334	341	+ 7	±30	
14	4 91	48 5	50 4	7 58	368	382	+14	±31	
24	4 91	49 2	50 4	6 95	342	350	+ 8	±31	
24	4 92	49 1	50 4	7 07	348	357	+ 9	±31	
8	4 92	55 7	50 4	7 95	443	401	−42	±32	−10
45	5 01	51 2	51 0	6 86	351	350	− 1	±31	
12	5 02	49 0	51 0	7 70	377	393	+16	±32	
40	5 03	51 6	51 1	7 03	363	359	− 4	±31	
1	5 06	52 3	51 3	6 86	359	352	− 7	±31	
21	5 16	53 0	51 8	7 55	400	391	− 9	±32	
29	5 18	53 6	52 0	8 26	443	429	−14	±32	
4	5 22	49 8	52 2	7 72	384	403	+19	±32	
34	5 32	54 0	52 8	7 96	430	420	−10	±32	
19	5 36	57 9	53 0	7 72	447	409	−38	±32	
13	5 44	52 2	53 5	8 07	421	432	+11	±33	
3	5 45	55 2	53 6	7 92	437	424	−13	±32	
53	5 48	54 2	53 7	7 82	424	420	− 4	±32	
17	5 49	54 6	53 8	8 54	467	459	− 8	±33	
24	5 55	52 1	54 1	8 11	423	439	+16	±33	
19	5 60	54 8	54 4	8 30	455	452	− 3	±33	
21	5 93	56 2	56 4	8 66	487	488	+ 1	±34	
13	5 94	49 6	56 4	8 70	432	491	+59	±34	+25
53	6 08	57 4	57 3	9 05	519	518	− 1	±34	
15	6 14	56 9	57 6	8 68	494	500	+ 6	±34	
34	6 62	59 2	60 5	9 64	571	583	+12	±36	

seen to be quite definitely linear, this fact being indicated clearly by the general appearance of the surface produced by the tips of the wires, which lay, in general, in a plane which was parallel to the G-axis and inclined to the A-axis These characteristics of the relationships are brought out in Figure 1, which shows the total lack of correlation between S and G and in Figure 2, which shows the high degree of correlation

TABLE II
Tabulation of errors of calculation and experimental errors

	Albumin	Total protein	Specific osmotic pressure	Total osmotic pressure
Variations of calculated from observed values				
Absolute variations				
			mm H₂O	*mm H₂O*
Standard error			± 2 23	
Average variation			± 1 74	±11 56
Maximum variations			+ 6 8	+59 0
			− 5 7	−42 0
Per cent variations				
			per cent	*per cent*
Standard deviation			± 5 05	± 5 05
Average per cent variation			± 3 87	± 3 89
Maximum per cent variations			+18 7	+18 4
			−13 1	−13 2
Experimental errors				
	grams per 100 cc	*grams per 100 cc*		*mm H₂O*
Differences in values of duplicate determinations				
Average difference	0 08	0 09		5 1
Maximum differences	0 39	0 66		19 0
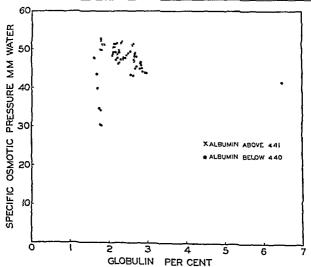
FIG 1   Showing complete lack of correlation between specific osmotic pressure and globulin concentration   Higher values of S correspond to high values of A, only and lower values of S correspond to lower values of A, only regardless of values of G
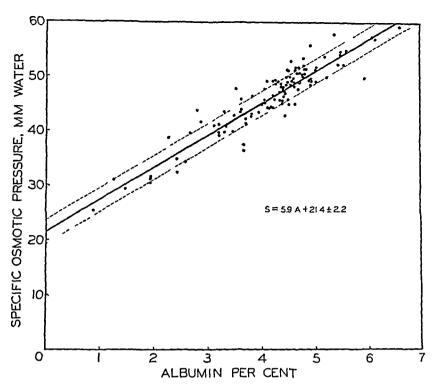
FIG 2   Showing the linear relation of specific osmotic pressure to albumin concentration, as a straight line, fitted by the method of least squares   Correlation coefficient of S and A is 0 93   Standard error of calculation, indicated by dotted lines, is ± 2 2 mm

between S and A   The Pearson coefficient of correlation between S and A was found to be 0 93, which indicates a high degree of relationship between the variables   The heavy line drawn through the points on the S–A graph is a straight line fitted to the data by the method of least squares   The equation of this line, $S = 5 9A + 21 4$ is corrected by means of the dotted lines on either side for the "standard error" of the calculated as compared to the observed values   The standard error being ± 2 2 mm of water, it may be prophesied on grounds of probability that, in any other similar series of measurements, the observed values of S will, in 68 per cent of the cases, fall within the range of values calculated from the formula $S = 5 9A + 21 4 ± 2 2$ Similarly, if the correction be doubled (twice the standard error being ± 4 4) the range will include 95 per cent of observed values, while if the correction be trebled 99 7 per cent of cases should be included   Actually, in our series the percentage of cases included by the corrections corresponding respectively to the standard error, to twice the standard error, and to three times the standard error, are 72 6, 93, and 98 5   The frequency distribution of the errors is therefore quite according to the "normal distribution "   The average
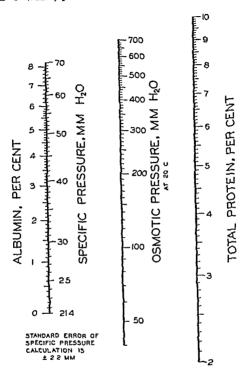
FIG 3 Nomogram   A straight line drawn through the proper points on the albumin and total protein scales will intersect the middle scale at the value of the total osmotic pressure calculated from the formula $P = C(21\,4 + 5\,9\,A)$

error of calculation of S was $\pm 1\,74$ mm and the maximum errors encountered were $+ 6\,8$ and $- 5\,7$ mm

The complete independence of the specific osmotic pressure from any effect of varying concentrations of globulin is exemplified by the findings on a sample of serum from the patient (Case Number 36) who suffered from Banti's disease The albumin concentration was 2 87 per cent and the globulin concentration was 6 48 per cent, giving an A/G ratio of 0 44 The observed specific osmotic pressure was 41 5 mm of water, while the value of S calculated from the albumin concentration is 38 3 On the assumption that the globulin fraction of serum exerts, universally, a lower osmotic pressure, gram for gram, than the albumin fraction, one

should expect the value of S of a serum having such an unusually high globulin content to be lower than that calculated by our formula   The fact that it was actually *higher*, by nearly 8 per cent, indicates clearly the unreliability of the A/G ratio as an index of the specific osmotic pressure, and confirms the idea that it is the albumin concentration which determines the specific osmotic properties of the serum

The total osmotic pressure, P, is calculated from the formula $P = C(5 \cdot 9A + 21 \cdot 4)$ The nomogram, reproduced in Figure 3, may be used to facilitate the calculations   The absolute error of the calculation of P is equal to the error of S multiplied by the concentration of total protein, C   The average error for our series was $\pm 11 \cdot 6$ mm , the maximum errors being $+ 59$ and $- 42$ mm   It is the percentage error of calculation of the total osmotic pressure, however, which will be most significant in practice   The percentage error will, in general, be the same for S as for P, and for our series the average per cent error is slightly less than $\pm 3 \cdot 9$, the maximum errors being $+ 18 \cdot 7$ and $- 13 \cdot 2$ per cent The standard deviation of the per cent errors about their mean (which is $- 0 \cdot 1$) is $\pm 5 \cdot 05$, which signifies that 68 out of every 100 values of S or P, calculated by the given formulae, will correspond to the observed values within approximately ± 5 per cent, and similarly 99 7 out of every 100 calculated values should check the observed values within ± 15 per cent   Actually, errors lying within the ranges ± 5 per cent and ± 15 per cent occurred in 77 and 99 per cent of cases, respectively The per cent errors of calculation may therefore be considered as being "normally" distributed

Sources of error

The term "observed value," as used in the last paragraph, refers, in each case, to the average of the two values obtained from duplicate osmotic pressure determinations   However, in 4 instances, due to a scarcity of serum, only a single determination was run, but in only 1 of these cases was the variation between the observed and calculated values as great as 5 per cent   The greatest variation between the two values obtained from duplicate determinations was 19 mm   The average variation was 5 1 mm   and 95 per cent of the cases showed variations which were not greater than 12 mm   In general there was no relation between the closeness of the check of the duplicate osmotic pressure determinations and the degree of variation of the observed mean pressure from the pressure calculated from the protein analyses

The question naturally arises as to what extent the variations of the calculated from the observed pressures may be accounted for on the basis of experimental errors and to what extent they must be considered as due to unknown factors   We should like to be able to decide to what extent the osmotic pressure of colloids of serum is due to the protein concentrations as we have measured them and to what extent it is due

to other factors that were not measured during these investigations The average variation of calculated from observed values was ± 11 5 mm while the average variation of duplicate osmotic pressure determinations was only 5 1 mm , or ± 2 55 mm when related to the means of the pairs of values Unfortunately the variation of duplicate determinations is not always a measure of the error of a method "Good checks," while reassuring, do not exclude the possibility that systematic errors may have affected both values to the same extent and in the same direction Allowing for the possibility that systematic errors were present of a type which could affect our pairs of observations now in one direction, now in another, at random, we should be fairly safe in assuming, arbitrarily, that the "maximum expected error" will not be greater than ± 12 mm , which is twice the variation from the mean of duplicate determinations in 95 per cent of our cases We can neglect the possibility that systematic errors may operate in one direction for the whole series of cases for they could not be responsible for variations of observed values from values calculated by a formula based on the same series of observations

The error of the chemical analysis of the albumin and total protein concentrations will contribute its share to the total error of the calculated value of the osmotic pressure In 93 per cent of the cases the variations between duplicate determinations of these quantities were not greater than 0 2 gram per 100 cc. Assuming that the maximum expected error is ± 0 2 gram per 100 cc for albumin and also for total protein, the maximum, additive error of both analyses will be responsible for a possible error of from ± 10 to ± 20 mm in the calculation of the osmotic pressure By the method of differentials, where the pressure is calculated by the formula $P = C(21\ 4 + 5\ 9A)$, the total error is the total differential, dP Now, $dP = 21\ 4dC + 5\ 9A\ dC + 5\ 9CdA$ Since dA and dC, the errors of the analyses, are both considered as ± 0 2, we have $dP = ± [1\ 2(A + C) + 4\ 3]$ The maximum error of analysis was calculated in this way for each case, and the maximum experimental error found by adding the maximum error of the osmotic pressure determination, which is considered to be ± 12 mm The figures are given in the last column of Table I Even with so great an allowance for errors of measurement, there were 6 instances in which the errors of calculation could not be accounted for It is therefore necessary to assume that other factors, which were not measured, have a determining influence upon the osmotic pressure exerted by a given sample of serum We may conclude that, whereas in a *series* of cases the errors due to the unknown factor or factors will not seriously affect the reliability of the calculation of the osmotic pressure from the albumin and total protein concentration, in any *single instance* the calculated value may possibly be in error by an amount which is much greater than the sum of the

experimental errors of the methods employed   The unknown factor does not seem to be referable to individual ("personal") peculiarities of the blood, for in no instance did a large error occur more than once in the series of determinations carried out on blood samples from individual subjects   It may be assumed that the method of calculation will give reliable results when applied to a series of determinations on the same individual as well as when applied to single determinations on a series of several individuals   As a matter of fact, the greatest error so far encountered, 19 per cent, is not so great as to discourage the employment of the method, for most clinical purposes, for single determinations on single individuals   In this respect the method cannot be considered to be less accurate than the usual determination of the basal metabolic rate, for which an error of $\pm$ 15 per cent is ordinarily allowed

The significance of the relation between the specific osmotic pressure and the total protein and albumin concentrations   It has been pointed out by Marrack and Hewitt (7) that the "Donnan pressure" due to the excess of diffusible ions in a protein solution over that in the outside solution will be proportional to the square of the protein concentration and must be added to the osmotic pressure of the protein alone in calculating the total osmotic pressure of an ideal protein solution   Theoretically, therefore, $P = aC + bC^2$, where a is the constant of proportionality relating the pressure due to the protein alone to the concentration, and where b is a constant relating to the Donnan pressure at constant pH   The equation, of course, will be expected to hold for a series of blood sera only if they contain the same proportions of the two protein fractions, albumin and globulin that is to say, when A/G remains constant   But when A/G is constant, C is directly proportional to A, so that our equation for the total osmotic pressure, $P = 21\ 4C + 5\ 9AC$, may be written $P = 21\ 4C + 5\ 9bC^2$, where b is the constant of proportionality between C and A for a constant value of A/G   This is the theoretical form of equation for the Donnan effect   Now $S = P/C$, so that the theoretical formula reduces to $S = a + bC$ For constant values of A/G our equation may be reduced to the identical form, $S = 21\ 4 + 5\ 9bC$

Interesting though these comparisons of our empirical equations with the theoretical Donnan equations may be, however, they do not completely explain the peculiar relations which we have found to exist   It is difficult to understand, on any theoretical basis which is not entirely speculative, why the specific pressure should be so completely independent of the globulin concentration and hence of the A/G ratio   The relation would seem to imply that the specific pressure of globulin in any serum is the same as that of the albumin with which it is associated, and that it has a value which is directly determined by the concentration of the albumin   On the other hand, globulin does not appear to have any effect on the specific pressure of albumin   It should be emphasized that

the term "specific pressure" refers not to the total osmotic pressure, but to the average pressure per gram per cent of total protein   The globulin content of a given serum appears to contribute gram for gram exactly as much to the total pressure as does the albumin   Consequently our data offer no justification for the assumption that albumin (in serum) has an effective molecular weight which is much less than that of globulin   The actual molecular weights of the two substances may, of course, be widely different but if they are, the fact is not reflected in our data and we must assume that other factors enter in to mask the effects of a difference in molecular weight   Whatever the explanation of this pe culiar situation may be, the existence of the relation indicates the need for an experimental study of the osmotic properties of both the separate and the mixed solutions of purified serum albumin and serum globulin

The finding that the albumin concentration determines the specific osmotic pressure, not only of the albumin itself but also of the accompanying globulin (regardless of the concentration of the globulin) is of some significance in relation to the clinical correlation of edema with low serum albumin concentrations for it becomes clear that a progressive lowering of the albumin content of a patient's serum entails a simul taneous lowering of the specific osmotic properties of the accompanying globulin fractions, even if the globulin concentration is not lowered

The significance of the A/G ratio as an index of the osmotic properties of serum depends, apparently, on the *clinical* fact that changes in this ratio are *most often* due to changes in the albumin fraction—the globulin changing relatively less, in most instances   However, in cases where the globulin undergoes marked variations from the normal value the A/G ratio becomes totally unreliable as an index   In our *series as a whole* there is a fair degree of correlation between the specific pressure and the A/G ratio, but this must be attributed solely to the particular selection of cases   In individual cases the correlation was often very poor indeed

The fact that the specific pressure increases with increasing total protein concentration (except when this increase is due to an increase in G alone) is in accord with the findings of many other investigators for serum, (8, 9, 10), for serum albumin (11), for other pure protein solutions (12, 13) and for all types of concentrated solutions of crystalloids so far studied, of which the classical example is sucrose (14)   Conse quently it would appear that the contention of Govaerts that the total protein concentration is without influence on the specific osmotic pressure is without justification except from his own data

The apparent unreliability of the formulae of Govaerts and of von Farkas In general the values obtained by Govaerts for the osmotic pressure of serum tend to be higher than those obtained by others   Further evidence that the values upon which Govaerts' formula is based are unreliable is given by the fact that Verney (8), employing cellophane membranes

furnished by Govaerts, obtained values which were lower in relation to the protein concentrations than those of Govaerts, but of the same order of magnitude as those of most other workers

We have used the formula of Govaerts, $P = 75\,4A + 19\,5G$, to calculate the osmotic pressures of our sera and find that these calculated values are, on the average, 18 4 per cent higher than the values which we observed, with a range of variation of $- 3$ to $+ 59$ per cent Since the formula of Govaerts fits his own data very well, we may conclude from the degree of variability found by the above-mentioned calculations that his observed pressures, mostly higher than ours, are not higher in any definite proportion but in a totally irregular manner If our determinations are correct, as we believe them to be, it follows that Govaerts' formula cannot be relied upon to give calculated values which are even *relatively* accurate The same criticism may be applied to the formula of von Farkas, $P = 68A + 25\,1G$, for the application of this formula to our data provides values which are, on the average, 13 4 per cent higher than the values observed by us, the range of variation being $- 8$ to $+ 56$ per cent

SUMMARY

1 The osmotic pressure of colloids and the total protein, albumin and globulin concentrations of 128 samples of human serum were determined The samples were obtained from 53 individuals

2 Analysis of the data, which cover a relatively wide range of values, indicates that the relation between the total protein and albumin concentrations and the osmotic pressure is sufficiently regular and definite to allow the use of an empirical formula, derived from the data, for the calculation of the osmotic pressure The formula is $P = C\,(21\,4 + 5\,9A)$, where P is the osmotic pressure in millimeters of water, C is the total protein concentration and A is the albumin concentration, in grams per 100 cc The standard error of calculation is ± 5 per cent

3 A nomogram has been constructed to facilitate calculations

4 The specific osmotic pressure of serum appears to be a linear function of the albumin concentration Variations in the globulin concentration over a wide range produce no effect on the specific pressure at constant values of A Consequently the A/G ratio is a poor index of the osmotic properties of serum The specific pressure increases for increasing values of the total protein concentration, but only in so far as these increases are not due to increases in the globulin The theoretical significance of these relations is discussed

5 The formulae of Govaerts and of von Farkas, which have been used to calculate the osmotic pressure, appear to be totally unreliable The reasons for this conclusion are discussed

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HERPETIC PHARYNGITIS AND STOMATITIS
A REPORT OF THREE CASES

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In recent years great interest has been manifested in the virus of herpes simplex It has been stimulated by an increasing curiosity regarding filterable viruses in general, and by the possibility that herpes virus may have a causal relationship to epidemic encephalitis However, despite the enthusiasm for the study of the properties of this filterable agent, little attention has been paid to the natural manifestations of the disease herpes simplex This may be due to the ubiquity of herpes simplex or it may be possible that the essential herpetic character of many affections is not recognized

The recognition of herpes as a clinical entity is probably of great antiquity, the word itself being derived from the Greek ἕρπης meaning literally "a creeping " Aretaeus the Cappadocian, in discussing affections of the tonsils, describes small superficial ulcers to which he gave the name aptha It is not unlikely that these lesions were herpetic in nature In 1398, John Trevisia in his fragmentary translation of Bartholomaeus Anglicus' manuscript, "On the Property of Things," makes mention of "this euyll is callyd Herpes" and "suche a scabbe highte Herpes Cingula " This constitutes the first reference to herpes in the English language, and it is plain from this citation that herpes was recognized in the Middle Ages During the succeeding centuries one finds a constant increase in the number of references to herpetic infections, thereby demonstrating the interest attached to the disease

However, it was not until the beginning of the nineteenth century that a clear differentiation was established between the clinical course of herpes simplex and herpes zoster Following this distinction one finds an increased interest taken by clinicians in the delineation of the various simple herpetic affections The general manifestations of the disease as well as the local, occupied their attention and in the middle of the century, several splendid clinical reports describing the disease are to be found

Gubler (1) in 1858 gives an admirable account of herpetic infections of the throat which we will quote "A la suite d'un refroidissement, un sujet est pris de malaise, de courbature, puis d'une fièvre quelquefois

assez intense, ainsi que d'un mal de gorge les deux amygdales sont tuméfiées, rouges et ne tardent par à présentes des surfaces circulaires ou irrégulièrement configurées, semblables à des ulcérations superficiells couvertes d'une exsudation plastique grisâtre ou jaunâtre, souvent cette dernière lésion est unilatérale En même temps il apparaît sur les lèvres une éruption d'herpès ordinairement groupée en grand partie vers l'une des commissures Tel est aussui le type consacré par le description classique Eh bien, dans mon opinion, ces ulcérations tonsillaires doivent être considérées comme de l'herpès labialis sur une surface muqueuse "

Trousseau (2) devoted an entire clinic to the description of herpetic pharyngitis His description of the individual lesions is classical "On voit en effet des taches blanche, entourées d'une aréole inflammatoire assez étendue, et dont le volume varie depuis le groisseur d'un grain de millet jus qu' à celui d'un pois Ces taches laissent à leur place des ulcérations superficielles dont les bords peuvent néanmoins être saillants en raison du gonflement oedémateux des tissus environnants envahis par l'inflammation "

In 1881 James Wilson in his book on "The Continued Fevers" describes simple continued fever as "a fever not due to any specific cause, usually of short duration, lacking the distinguishing. characteristics of other fevers, and rarely fatal in temperate climates The eruption of herpes upon the lips and nose is so common at the close of simple continued fever that this disease by some persons has been called Herpetic Fever "

Two years later Savage (3) reported an epidemic of herpetic fever which occurred in an English boys' school The clinical picture of the disease in this epidemic was characterized by a slow onset accompanied by headache, lassitude, chilliness, fever, irritability, malaise and vomiting After twenty-four hours, patches of herpetic lesions appeared about the lips or lobes of the ears In two individuals vesicles appeared on the lower extremities With the appearance of the vesicles the patients complained of sore throats Frequently herpetic lesions were present in the throat By the fourth day the temperature was normal and the patients were convalescent Thirty-nine boys were affected during a two-week period

In recent years, despite Trousseau's remark that "Au jour d'hui qu' on est suffisamment averti, il est peu de médecins qui n'aient en l'occasion d'en observer des exemples," very little can be found in medical literature regarding the clinical course of localized and generalized herpetic infections of the simplex type In 1932 Youmans (4) described an individual ill from herpetic fever with stomatitis He was successful in isolating the virus of herpes simplex from the mouth lesions in this patient, thus for the first time demonstrating the essential etiological character of the disease

In this report we wish to discuss three cases of herpetic infections in the mouth, two of which were accompanied by fever   From two of the individuals the virus of herpes simplex was recovered

CASE HISTORIES

Miss C K J H H 40611, a student aged 23 entered the Medical Clinic on December 9, 1931, complaining of a "sore throat and swollen tender gums' of three days duration   Her past and family history was unimportant   Three days before coming to the clinic the patient had spent the week end in New York and while riding to Baltimore on the train, she noticed that her throat was sore   Upon the following day she experienced "chills and fever "   The soreness in the throat increased and the gums became tender and swollen   Headache appeared and some generalized aching was present   On December 8th the symptoms were more marked and the throat affection was so severe that swallowing became very difficult   She was admitted to the Isolation Ward on the following morning

Physical examination   The patient was undernourished and seemed quite uncomfortable   The temperature was 101 6° F , the pulse 112, and the respirations were 20   The skin was warm and moist the ears, eyes and nose were normal   The lips were dry and cracked and just to the right of the midline was a small, crusted, superficial ulcerated lesion   The gums were tender, swollen and spongy and there were numerous small superficial lesions capped with white membranes on the tooth gum margins   These bled freely when slightly traumatized   Near the tip of the tongue were several small vesicles as well as upon the hard palate   The tonsils had been cleanly removed, but the soft palate, uvula pillars and posterior pharyngeal wall were intensely inflamed and moderately swollen   The lymphoid follicles of the posterior pharyngeal wall were markedly hypertrophied and there were numerous small superficial vesicular lesions over the posterior pharyngeal wall   The regional lymph nodes were enlarged and slightly tender as were also the axillary glands   The remainder of the physical examination was essentially normal

Laboratory data   Urine normal   Red blood cells 4 4 million   Hemo globin 90 per cent   White blood cells 4,560   Differential count—Poly morphonuclears 72 5 lymphocytes 12 5, monocytes 15   Wassermann reaction negative   Throat cultures (7), staphylococcus aureus   Throat smears—no Vincent s organisms   Blood culture negative

Impression   It was thought on admission to the Ward that the patient was suffering from an acute septic sore throat and an aphthous stomatitis   The possibility of a Vincent s infection was considered, but at no time were Vincent s organisms demonstrated

Course   The temperature remained elevated on the day after admission and the total white blood cells numbered 6 000   The patient complained of a severe sore throat   The mouth lesions were unchanged   On December 11th the temperature was lower and the white blood cells totaled 5 950   The white membranes capping the vesicles were beginning to slough off leaving clean superficial ulcers   On December 12th the temperature fell to normal and there was a marked symptomatic improvement   White blood cells 3 750   Treatment of the ulcers by topical application of 2 per cent gentian violet was instituted   During the next few days the patient improved and by December 18th her mouth and throat were free from ulcerated areas   On December 22nd she was discharged well, and as far as we know she has never had a recurrence of this affection

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Mr M T , J H H 46235, a white, night club saxophone player, aged 25 years, entered the Medical Clinic on November 4th, 1932, complaining of a "sore mouth" of six days duration   His past and family history was unimportant   Five days before coming to the hospital the patient felt "feverish" and thought that his tongue and gums were dry and swollen   A moderately severe headache was present   By evening he developed definite soreness of the throat, tongue and gums   On the following day he felt "grippy" and suffered from generalized aching and malaise   By the next day his throat was so sore and he felt so seedy that he took to his bed   Profuse sweats occurred, his neck became swollen and tender and his headaches more severe   He suffered from insomnia and on the night before entry he was disoriented   All of his symptoms increased in intensity and he came to the hospital

Physical examination   The patient was well developed and well nourished, but apparently was very uncomfortable   The temperature was 100° F , the pulse 96, and the respirations 22   The skin was hot and dry, the ears, eyes and nose were normal   The lips were dry, fissured and scaly   Several small superficial crusted ulcers were present on the outer dry surface of the lips   The inner surface of the lips, the buccal mucous membranes, and the gums were dull red in color   Many deflated vesicles capped with dead white skin were seen on these surfaces   The gums bled when slightly traumatized   The tongue was beefy red and was covered with numerous vesicles   Many similar lesions were seen on the hard palate   The soft palate, uvula, pillars, and posterior pharyngeal wall were moderately inflamed and slightly swollen   On the soft palate, uvula and right posterior pillar there were small vesicles surrounded by a small area of intense hyperemia and capped with a dull chalk-white tense membrane   These collapsed completely when the vesicular fluid was released   The regional and anterior cervical lymph nodes were enlarged and tender   Otherwise the physical examination was normal

Laboratory data   Urine normal   Red blood cells 4 6 million   Hemoglobin 106   White blood cells 6,880   Differential count—polymorphonuclears 62, eosinophiles 1, basophils 1, lymphocytes 34, monocytes 2   Wassermann reaction negative   Throat smears—no Vincent's organisms   Throat culture —staphylococcus aureus

Impression   Because of the presence of typical herpetic lesions upon the lips and the vesicular character of the eruptions in the mouth, it was thought that this patient was suffering from herpetic stomatitis and pharyngitis

Course   On the day following the patient's admission to the hospital, his temperature was normal, but there was no change in the condition of the mouth   The mouth lesions were treated with topical applications of gentian violet   On November 8th the white cells numbered 7,700   The lesions were slowly resolving, but enough discomfort persisted to prevent the taking of solid food   By November 11th the lesions had practically disappeared and the patient was discharged   There has been no recurrence of the herpes up to the present time

Miss C W , J H H 46136, a student nurse, aged 22, entered the Medical Clinic on January 2nd, 1933, complaining of a "sore throat" of two days duration   With the onset of this affection she suffered from "chills and fever," headache and anorexia   On the following day she felt weak and experienced generalized aching   Profuse sweats occurred during the night   On the day of admission malaise was present and the glands in her neck were enlarged and tender

Physical examination   The patient was well nourished and seemed com-

fortable   The temperature, pulse and respirations were normal   A faint, generalized erythema was present   The ears, eyes and nose were normal, as were also the lips gums, buccal mucous membranes and tongue   A discrete vesicular eruption was present over the left anterior pillar and over a right tonsillar tag   The vesicles ranged in size from two to four millimeters and were surrounded by small areas of very hyperemic mucous membrane   The entire throat was mildly erythematous and the lymphoid follicles on the posterior pharyngeal wall were hypertrophied   The regional lymphnodese were moderately enlarged and tender   Otherwise the physical examination was normal

Laboratory data   Urine normal   Red blood cells 4 9 million   Hemoglobin 100   White blood cells 7,050   Differential count—polymorphonuclears 65, eosinophiles 1   basophils 1, lymphocytes 29, monocytes 4   Wassermann reaction negative   Throat culture—staphylococcus albus and aureus

In pression   Herpetic pharyngitis

Course   The temperature, pulse and respirations remained normal through out the patient s stay in the hospital   By January 6th the throat had improved and on the following day the patient was discharged from the hospital   No local treatment was used in this instance   There has not been any recurrence of the affection to date

Experimental studies

Miss C K   No studies were made because the nature of the throat affection was not suspected until after the vesicles had disappeared

Mr M T   Upon November 4th, the cap of a tense vesicle was removed with biting forceps and this material was rubbed into the scarified left corneae of two rabbits   Within twenty four hours a localized injection of the sclera was observed and after a short time one could see minute vesicles along the lines of scarification with the aid of a hand lens   By November 7th a full blown, steamy keratitis was manifest in both animals   At this point, rabbit Number 1 was killed, the left cornea was removed and then ground in a sterile mortar with a small amount of sterile physiological saline solution   The left scarified corneae of rabbits Numbers 3 and 4 were inoculated with the ground material

On the 12th of November rabbit Number 2 was hypersensitive and circled definitely to the left   Three days later this animal was extremely hypersensitive, circled to the left, showed marked salivation and retention of urine, ran blindly into objects and had an accelerated respiratory rate   Its temperature was 106 8° F   The rabbit was killed and its brain carefully removed with sterile precautions   A part of the brain was placed in Zenker s acetic fluid, a second portion was cultured in aerobic and anaerobic media, and the remaining bit was ground up in sterile physiological saline solution and the brain suspension was inoculated into the scarified left cornea of a guinea pig   This animal developed a typical keratitis and on the fourth day after inoculation showed characteristic signs of encephalitis   It was then killed and its brain was placed in Zenker's acetic fluid

Rabbits Numbers 3 and 4 both developed keratitis within twenty-four hours of inoculation and showed definite signs of encephalitis on the seventh day   At that time they were killed and their brains were placed in Zenker's acetic fixing fluid   Histological sections stained with Mallory's eosin-methylene blue stain were made of the fixed tissues   Examination of these sections showed in each instance the severe, infiltrative meningoencephalitis and the acidophilic intranuclear inclusions which are characteristic of herpetic encephalitis in laboratory animals

Miss C W   On January 4th, the top of a tense vesicle was removed and was rubbed on the scarified cornea of a rabbit   By January 6th a definite keratitis was present and on the following day the animal was killed and the infected cornea removed   Part of this cornea was placed in Zenker's acetic fluid and the remainder was used to inoculate the scarified cornea of a second rabbit   This animal developed keratitis within two days and showed signs of encephalitis on the eighth day, at which time it was killed and its brain placed in Zenker's acetic fluid   Histological sections stained with Mallory's eosin-methylene blue stain were made of the fixed tissues   The cornea of the first rabbit showed a severe infiltrative keratitis with rare intranuclear inclusion bodies   Evidently the process had progressed beyond the stage most favorable for the finding of inclusion bodies   A severe meningoencephalitis was present in the second rabbit and numerous brain cells showed the acidophilic intranuclear inclusion bodies which are considered to be typical of herpetic infection

The clinical course of herpetic stomatitis and pharyngitis

In all of our cases the onset was slow and the first symptom observed was either a sore mouth or sore throat   Shortly after the onset, headache and malaise accompanied by sensation of "chills and fever" appeared   Sweating was often marked   It was not possible in any of our cases to determine the relation of the appearance of the herpetic lesions to the onset of symptoms   Nausea and vomiting did not occur   As the disease progressed the symptoms became more intense   In all three individuals generalized aching was present at one time or the other   Two patients had a definite fever when admitted to the Ward   These two also had labial herpes and the lesions were widespread in their mouths   None of our cases developed skin lesions   All had white blood cell counts below 8,000 on entry   The differential formulae were normal   Treatment other than palliative seemed to be of little value as the disease is self limited   None of our patients gave a history of susceptibility to herpes and none have had a recurrence of the infection

We were able to isolate a virus which produced the typical clinical syndromes of herpetic keratitis and encephalitis in rabbits and guinea

pigs from the vesicles in two of three patients   Histological preparations
of the corneae and brains from the infected animals showed the acidophilic
intranuclear inclusions which are considered to be characteristic of
herpetic infections

It is very likely that herpetic affections of the mouth and throat are
more common than we suspect   It seems curious that a disease appa-
rently so well known and so frequently recognized in the last century
should have become rare   It is probable that if more attention is paid
to the clinical appearance of "sore throats," many of them will be
recognized as being herpetic in origin

SUMMARY

Three cases of herpetic pharyngitis and stomatitis have been de-
scribed and discussed   A virus corresponding to the virus of herpes
simplex was isolated from the lesions in two of the three cases

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STUDIES UPON THE NASAL SECRETIONS I THE CELLULAR CONTENT OF THE NASAL SECRETIONS IN ACUTE DISEASE OF THE RESPIRATORY TRACT

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Although common colds are probably the most frequent ailments from which we suffer, very few studies have been made upon the changes that take place in the nasal mucous membranes during acute upper respiratory infections In 1885 MacKenzie (1) first described the histological appearance of the nasal mucous membranes during acute coryza He found an intense engorgement of the cavernous tissue which was especially well marked over the lower halves of the middle and posterior portions of the inferior turbinates with rupture of the vessels Along the inner walls of the dilated spaces were congregations of lymphoid cells and in some of them collections of fibrinous exudate A moderate cellular infiltration was present below the basement membrane

Forty five years after this report, Hilding (2) published his observations upon the histopathology of the nasal mucous membranes in "colds" He found that "the pathologic process is that of a mucous membrane inflammation showing rather marked tissue changes, including the loss of many of the surface cells and a proliferative reaction in the submucosa The epithelium is regenerated and repaired by the growth and multiplication of the stellate cells normally found deep in the epithelium "

While studying the nasal secretions, Hilding noted that the cellular content changed rapidly Early in colds the secretion was watery and contained few cells Ciliated epithelial cells appeared during the first twenty four hours and became more numerous by the second day The deeper epithelial cells, polyblasts and polymorphonuclear cells appeared in the secretions on the second day and rapidly became numerous Within a few days the polymorphonuclears made up most of the cellular content of the secretions

Hilding did not believe that the cellular content of the secretions or the pathological picture in the nasal mucous membranes was related to any one causal agent in colds, but interpreted the observed changes as a response to any one of or a combination of etiological factors

D and R Thomson (3) have briefly described nasal secretions from cases of acute watery catarrh Their observations are of interest chiefly

assez intense, ainsi que d'un mal de gorge les deux amygdales sont tuméfiées, rouges et ne tardent par à présentes des surfaces circulaires ou irrégulièrement configurées, semblables à des ulcérations superficiells couvertes d'une exsudation plastique grisâtre ou jaunâtre, souvent cette dernière lésion est unilatérale En même temps il apparaît sur les lèvres une éruption d'herpès ordinairement groupée en grand partie vers l'une des commissures Tel est aussui le type consacré par le description classique Eh bien, dans mon opinion, ces ulcérations tonsillaires doivent être considérées comme de l'herpès labialis sur une surface muqueuse "

Trousseau (2) devoted an entire clinic to the description of herpetic pharyngitis His description of the individual lesions is classical "On voit en effet des taches blanche, entourées d'une aréole inflammatoire assez étendue, et dont le volume varie depuis le groisseur d'un grain de millet jus qu' à celui d'un pois Ces taches laissent à leur place des ulcérations superficielles dont les bords peuvent néanmoins être saillants en raison du gonflement oedémateux des tissus environnants envahis par l'inflammation "

In 1881 James Wilson in his book on "The Continued Fevers" describes simple continued fever as "a fever not due to any specific cause, usually of short duration, lacking the distinguishing characteristics of other fevers, and rarely fatal in temperate climates The eruption of herpes upon the lips and nose is so common at the close of simple continued fever that this disease by some persons has been called Herpetic Fever "

Two years later Savage (3) reported an epidemic of herpetic fever which occurred in an English boys' school The clinical picture of the disease in this epidemic was characterized by a slow onset accompanied by headache, lassitude, chilliness, fever, irritability, malaise and vomiting After twenty-four hours, patches of herpetic lesions appeared about the lips or lobes of the ears In two individuals vesicles appeared on the lower extremities With the appearance of the vesicles the patients complained of sore throats Frequently herpetic lesions were present in the throat By the fourth day the temperature was normal and the patients were convalescent Thirty-nine boys were affected during a two-week period

In recent years, despite Trousseau's remark that "Au jour d'hui qu' on est suffisamment averti, il est peu de médecins qui n'aient en l'occasion d'en observer des exemples," very little can be found in medical literature regarding the clinical course of localized and generalized herpetic infections of the simplex type In 1932 Youmans (4) described an individual ill from herpetic fever with stomatitis He was successful in isolating the virus of herpes simplex from the mouth lesions in this patient, thus for the first time demonstrating the essential etiological character of the disease

In this report we wish to discuss three cases of herpetic infections in the mouth, two of which were accompanied by fever   From two of the individuals the virus of herpes simplex was recovered

CASE HISTORIES

Miss C K J H H 40611, a student, aged 23 entered the Medical Clinic on December 9, 1931, complaining of a "sore throat and swollen tender gums' of three days duration   Her past and family history was unimportant   Three days before coming to the clinic the patient had spent the week end in New York and while riding to Baltimore on the train, she noticed that her throat was sore   Upon the following day she experienced "chills and fever "   The soreness in the throat increased and the gums became tender and swollen   Headache appeared and some generalized aching was present   On December 8th the symptoms were more marked and the throat affection was so severe that swallowing became very difficult   She was admitted to the Isolation Ward on the following morning

Physical examination   The patient was undernourished and seemed quite uncomfortable   The temperature was 101 6° F , the pulse 112, and the respirations were 20   The skin was warm and moist the ears, eyes and nose were normal   The lips were dry and cracked and just to the right of the midline was a small, crusted, superficial ulcerated lesion   The gums were tender, swollen and spongy and there were numerous small superficial lesions capped with white membranes on the tooth gum margins   These bled freely when slightly traumatized   Near the tip of the tongue were several small vesicles as well as upon the hard palate   The tonsils had been cleanly removed, but the soft palate, uvula pillars and posterior pharyngeal wall were intensely inflamed and moderately swollen   The lymphoid follicles of the posterior pharyngeal wall were markedly hypertrophied and there were numerous small superficial vesicular lesions over the posterior pharyngeal wall   The regional lymph nodes were enlarged and slightly tender as were also the axillary glands   The remainder of the physical examination was essentially normal

Laboratory data   Urine normal   Red blood cells 4 4 million   Hemo globin 90 per cent   White blood cells 4,560   Differential count—Poly morphonuclears 72 5 lymphocytes 12 5, monocytes 15   Wassermann reaction negative   Throat cultures (7), staphylococcus aureus   Throat smears—no Vincent s organisms   Blood culture negative

Impression   It was thought on admission to the Ward that the patient was suffering from an acute septic sore throat and an aphthous stomatitis   The possibility of a Vincent s infection was considered, but at no time were Vincent's organisms demonstrated

Course   The temperature remained elevated on the day after admission and the total white blood cells numbered 6 000   The patient complained of a severe sore throat   The mouth lesions were unchanged   On December 11th the temperature was lower and the white blood cells totaled 5,950   The white membranes capping the vesicles were beginning to slough off, leaving clean superficial ulcers   On December 12th the temperature fell to normal and there was a marked symptomatic improvement   White blood cells 3 750   Treatment of the ulcers by topical application of 2 per cent gentian violet was instituted   During the next few days the patient improved and by December 18th her mouth and throat were free from ulcerated areas   On December 22nd she was discharged well, and as far as we know, she has never had a recurrence of this affection

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Mr M T , J H H 46235, a white, night club saxophone player, aged 25 years, entered the Medical Clinic on November 4th, 1932, complaining of a "sore mouth" of six days duration   His past and family history was unimportant   Five days before coming to the hospital the patient felt "feverish" and thought that his tongue and gums were dry and swollen   A moderately severe headache was present   By evening he developed definite soreness of the throat, tongue and gums   On the following day he felt "grippy" and suffered from generalized aching and malaise   By the next day his throat was so sore and he felt so seedy that he took to his bed   Profuse sweats occurred, his neck became swollen and tender and his headaches more severe   He suffered from insomnia and on the night before entry he was disoriented   All of his symptoms increased in intensity and he came to the hospital

Physical examination   The patient was well developed and well nourished, but apparently was very uncomfortable   The temperature was 100° F , the pulse 96, and the respirations 22   The skin was hot and dry, the ears, eyes and nose were normal   The lips were dry, fissured and scaly   Several small superficial crusted ulcers were present on the outer dry surface of the lips   The inner surface of the lips, the buccal mucous membranes, and the gums were dull red in color   Many deflated vesicles capped with dead white skin were seen on these surfaces   The gums bled when slightly traumatized   The tongue was beefy red and was covered with numerous vesicles   Many similar lesions were seen on the hard palate   The soft palate, uvula, pillars, and posterior pharyngeal wall were moderately inflamed and slightly swollen   On the soft palate, uvula and right posterior pillar there were small vesicles surrounded by a small area of intense hyperemia and capped with a dull chalk-white tense membrane   These collapsed completely when the vesicular fluid was released   The regional and anterior cervical lymph nodes were enlarged and tender   Otherwise the physical examination was normal

Laboratory data   Urine normal   Red blood cells 4 6 million   Hemoglobin 106   White blood cells 6,880   Differential count—polymorphonuclears 62, eosinophiles 1, basophils 1, lymphocytes 34, monocytes 2   Wassermann reaction negative   Throat smears—no Vincent's organisms   Throat culture —staphylococcus aureus

Impression   Because of the presence of typical herpetic lesions upon the lips and the vesicular character of the eruptions in the mouth, it was thought that this patient was suffering from herpetic stomatitis and pharyngitis

Course   On the day following the patient's admission to the hospital, his temperature was normal, but there was no change in the condition of the mouth   The mouth lesions were treated with topical applications of gentian violet   On November 8th the white cells numbered 7,700   The lesions were slowly resolving, but enough discomfort persisted to prevent the taking of solid food   By November 11th the lesions had practically disappeared and the patient was discharged   There has been no recurrence of the herpes up to the present time

Miss C W , J H H 46136, a student nurse, aged 22, entered the Medical Clinic on January 2nd, 1933, complaining of a "sore throat" of two days duration   With the onset of this affection she suffered from "chills and fever," headache and anorexia   On the following day she felt weak and experienced generalized aching   Profuse sweats occurred during the night   On the day of admission malaise was present and the glands in her neck were enlarged and tender

Physical examination   The patient was well nourished and seemed com-

fortable   The temperature, pulse and respirations were normal   A faint, generalized erythema was present   The ears, eyes and nose were normal, as were also the lips gums, buccal mucous membranes and tongue   A discrete vesicular eruption was present over the left anterior pillar and over a right tonsillar tag   The vesicles ranged in size from two to four millimeters and were surrounded by small areas of very hyperemic mucous membrane   The entire throat was mildly erythematous and the lymphoid follicles on the posterior pharyngeal wall were hypertrophied   The regional lymphnodese were moderately enlarged and tender   Otherwise the physical examination was normal

Laboratory data   Urine normal   Red blood cells 4 9 million   Hemoglobin 100   White blood cells 7,050   Differential count—polymorphonuclears 65, eosinophiles 1 basophils 1, lymphocytes 29, monocytes 4   Wassermann reaction negative   Throat culture—staphylococcus albus and aureus

Impression   Herpetic pharyngitis

Course   The temperature, pulse and respirations remained normal through out the patient s stay in the hospital   By January 6th the throat had improved and on the following day the patient was discharged from the hospital   No local treatment was used in this instance   There has not been any recurrence of the affection to date

Experimental studies

Miss C K   No studies were made because the nature of the throat affection was not suspected until after the vesicles had disappeared

Mr M T   Upon November 4th, the cap of a tense vesicle was removed with biting forceps and this material was rubbed into the scarified left corneae of two rabbits   Within twenty four hours a local-ized injection of the sclera was observed and after a short time one could see minute vesicles along the lines of scarification with the aid of a hand lens   By November 7th a full blown, steamy keratitis was manifest in both animals   At this point, rabbit Number 1 was killed, the left cornea was removed and then ground in a sterile mortar with a small amount of sterile physiological saline solution   The left scarified corneae of rabbits Numbers 3 and 4 were inoculated with the ground material

On the 12th of November rabbit Number 2 was hypersensitive and circled definitely to the left   Three days later this animal was extremely hypersensitive, circled to the left, showed marked salivation and retention of urine, ran blindly into objects and had an accelerated respiratory rate Its temperature was 106 8° F   The rabbit was killed and its brain care-fully removed with sterile precautions   A part of the brain was placed in Zenker s acetic fluid, a second portion was cultured in aerobic and anaerobic media, and the remaining bit was ground up in sterile physio logical saline solution and the brain suspension was inoculated into the scarified left cornea of a guinea pig   This animal developed a typical keratitis and on the fourth day after inoculation showed characteristic signs of encephalitis   It was then killed and its brain was placed in Zenker's acetic fluid

Rabbits Numbers 3 and 4 both developed keratitis within twenty-four hours of inoculation and showed definite signs of encephalitis on the seventh day   At that time they were killed and their brains were placed in Zenker's acetic fixing fluid   Histological sections stained with Mallory's eosin-methylene blue stain were made of the fixed tissues   Examination of these sections showed in each instance the severe, infiltrative meningoencephalitis and the acidophilic intranuclear inclusions which are characteristic of herpetic encephalitis in laboratory animals

Miss C W   On January 4th, the top of a tense vesicle was removed and was rubbed on the scarified cornea of a rabbit   By January 6th a definite keratitis was present and on the following day the animal was killed and the infected cornea removed   Part of this cornea was placed in Zenker's acetic fluid and the remainder was used to inoculate the scarified cornea of a second rabbit   This animal developed keratitis within two days and showed signs of encephalitis on the eighth day, at which time it was killed and its brain placed in Zenker's acetic fluid   Histological sections stained with Mallory's eosin-methylene blue stain were made of the fixed tissues   The cornea of the first rabbit showed a severe infiltrative keratitis with rare intranuclear inclusion bodies   Evidently the process had progressed beyond the stage most favorable for the finding of inclusion bodies   A severe meningoencephalitis was present in the second rabbit and numerous brain cells showed the acidophilic intranuclear inclusion bodies which are considered to be typical of herpetic infection

The clinical course of herpetic stomatitis and pharyngitis

In all of our cases the onset was slow and the first symptom observed was either a sore mouth or sore throat   Shortly after the onset, headache and malaise accompanied by sensation of "chills and fever" appeared   Sweating was often marked   It was not possible in any of our cases to determine the relation of the appearance of the herpetic lesions to the onset of symptoms   Nausea and vomiting did not occur   As the disease progressed the symptoms became more intense   In all three individuals generalized aching was present at one time or the other   Two patients had a definite fever when admitted to the Ward   These two also had labial herpes and the lesions were widespread in their mouths   None of our cases developed skin lesions   All had white blood cell counts below 8,000 on entry   The differential formulae were normal   Treatment other than palliative seemed to be of little value as the disease is self limited   None of our patients gave a history of susceptibility to herpes and none have had a recurrence of the infection

We were able to isolate a virus which produced the typical clinical syndromes of herpetic keratitis and encephalitis in rabbits and guinea

pigs from the vesicles in two of three patients   Histological preparations
of the corneae and brains from the infected animals showed the acidophilic
intranuclear inclusions which are considered to be characteristic of
herpetic infections

It is very likely that herpetic affections of the mouth and throat are
more common than we suspect   It seems curious that a disease appa-
rently so well known and so frequently recognized in the last century
should have become rare   It is probable that if more attention is paid
to the clinical appearance of "sore throats," many of them will be
recognized as being herpetic in origin

SUMMARY

Three cases of herpetic pharyngitis and stomatitis have been de-
scribed and discussed   A virus corresponding to the virus of herpes
simplex was isolated from the lesions in two of the three cases

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STUDIES UPON THE NASAL SECRETIONS I THE CELLULAR CONTENT OF THE NASAL SECRETIONS IN ACUTE DISEASE OF THE RESPIRATORY TRACT

By PERRIN H LONG, ELEANOR A BLISS AND HARRIET M CARPENTER

(From the Laboratory of the John J Abel Fund for Research on the Common Cold and from the Department of Medicine Johns Hopkins University Baltimore)

Although common colds are probably the most frequent ailments from which we suffer, very few studies have been made upon the changes that take place in the nasal mucous membranes during acute upper respiratory infections   In 1885 MacKenzie (1) first described the histological appearance of the nasal mucous membranes during acute coryza He found an intense engorgement of the cavernous tissue which was especially well marked over the lower halves of the middle and posterior portions of the inferior turbinates with rupture of the vessels   Along the inner walls of the dilated spaces were congregations of lymphoid cells and in some of them collections of fibrinous exudate   A moderate cellular infiltration was present below the basement membrane

Forty five years after this report, Hilding (2) published his observations upon the histopathology of the nasal mucous membranes in "colds " He found that "the pathologic process is that of a mucous membrane inflammation showing rather marked tissue changes, including the loss of many of the surface cells and a proliferative reaction in the submucosa The epithelium is regenerated and repaired by the growth and multiplication of the stellate cells normally found deep in the epithelium "

While studying the nasal secretions, Hilding noted that the cellular content changed rapidly   Early in colds the secretion was watery and contained few cells   Ciliated epithelial cells appeared during the first twenty four hours and became more numerous by the second day   The deeper epithelial cells, polyblasts and polymorphonuclear cells appeared in the secretions on the second day and rapidly became numerous Within a few days the polymorphonuclears made up most of the cellular content of the secretions

Hilding did not believe that the cellular content of the secretions or the pathological picture in the nasal mucous membranes was related to any one causal agent in colds, but interpreted the observed changes as a response to any one of or a combination of etiological factors

D and R Thomson (3) have briefly described nasal secretions from cases of acute watery catarrh   Their observations are of interest chiefly

because of their description of certain round bodies which they saw in the cytoplasm of squamous epithelial cells   Attention is called to the resemblances of these bodies to the guarnieri bodies found within the epithelial cells in vaccinia and variola

During the past two years we have been studying the cellular content of the nasal secretions in the course of common colds in an attempt to determine the types of cellular response, the nature of the cells, and if possible, the underlying factors which govern the appearance of the various cellular constituents of the nasal secretions in acute upper respiratory tract disease   This communication describes our observations upon the types of cellular response found in the nasal secretions in disease of the upper respiratory tract

MATERIAL AND METHODS

The subjects utilized in this study were medical students and laboratory workers in the Johns Hopkins University Medical School   A definite attempt was made to obtain the nasal secretions at the beginning and at frequent intervals during the course of the colds   The secretions were gained by having the subject blow his nose upon a clean piece of non-absorbent brown paper and from the nasal discharge preparations were made upon glass slides covered with dried neutral red and Janus green for "supra vital" studies   Permanent preparations were made by evenly smearing the discharge upon clean glass slides and permitting it to dry in the air   The secretions were also observed as to color, consistency and amount

The "supra vital" preparations were examined immediately in a warm box at 37° C and the percentage of the different types of cells was determined   The air dried smears were subsequently stained with thionin blue and were studied for the presence of bacteria and bacterial phagocytosis   Other air dried slide preparations were stained with either Wright's stain or Mallory's eosin-methylene blue stain and were studied for cell types and for the possible presence of intracellular inclusion bodies   No attempt was made to determine the total number of cells in the specimens of nasal secretions

We have had the opportunity of examining 302 specimens of nasal secretions in the various stages of 98 colds   The majority of the examinations were made in the first week of the infection   We have been unable to obtain what we considered to be normal nasal secretions for control studies, so we have therefore used the nasal discharge from individuals suffering from acute hay fever to control our observations upon the secretions obtained from persons ill with "colds "

EXPERIMENTAL OBSERVATIONS

In the early stages of colds one commonly finds polymorphonuclear leukocytes, macrophages (monocytes and clasmatocytes), squamous and

columnar epithelial cells, eosinophilic leukocytes, lymphocytes and red blood corpuscles present in the nasal secretions   The cellular response (in the nasal discharge) during the first forty eight hours of a cold may be of three types   In the first type the majority of the cells are macrophages and epithelial cells, in the second type polymorphonuclear leukocytes, and in the third eosinophilic leukocytes   Striking examples of these three types are not commonly encountered as most of the samples of early nasal secretions showed cellular contents which were gradations between the extremes   The three types of response together with the average are shown in Charts 1, 2, 3 and 4
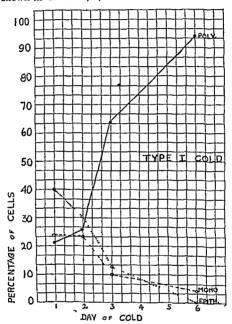
CHART I   A COMMON COLD SHOWING A PRIMARY MACROPHAGE EPITHELIAL
CELL RESPONSE IN THE NASAL SECRETIONS

When the macrophage epithelial cell type of response is present the secretions contain many macrophages and epithelial cells   The macrophages are easily distinguished in the "supra vital" preparations, but at times the classification of the epithelial cells is difficult because of the bizarre forms which are seen   It is common to find the upper half of the

cells pinched off and then one sees curious globular ciliated cells which resemble bursting grenades   Again one may observe elongated, rounded end, finely granular cells with no visible nuclear structure which may or may not contain vacuoles holding the neutral red and which may or may not have cilia   We think that these cells represent degenerated ciliated columnar epithelial cells   The amount of neutral red taken in by the columnar cells varies from none to 7 or 8 vacuoles filled with this
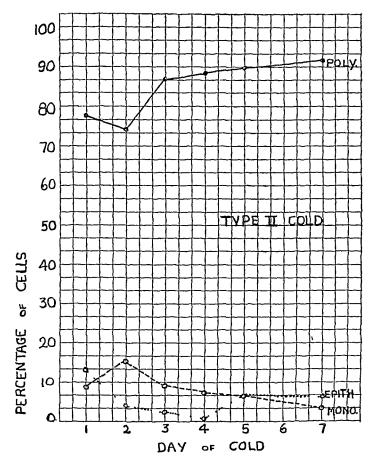
CHART II   A COMMON COLD SHOWING A PREDOMINANT POLYMORPHONU-
CLEAR LEUKOCYTE RESPONSE IN THE NASAL SECRETIONS

dye   The recognition of the squamous epithelial cells from the posterior rhinopharynx and vestibule offers no difficulties

An evaluation of the viability of the ciliated columnar epithelial cells upon the basis of motility of the cilia and staining of the nucleus has been attempted without much success   In many preparations the cilia were highly motile, in others non-motile   We suspect that in most instances these cells are alive in the secretions, but that due to unavoid-

able crushing during the preparation of the supra vital slide the cilia are so injured that their lack of motility loses its value as a criterion of viability

The macrophages generally appeared to be alive and always showed various amounts of phagocyted material We were unable to determine the exact mechanism which regulated the degree of phagocytosis, but we believe that it depended upon the amount of cellular debris present
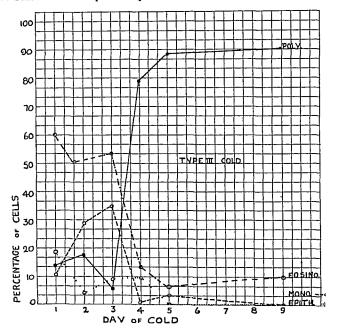
CHART III   A COMMON COLD SHOWING A PRIMARY EOSINOPHILE LEUKOCYTE RESPONSE IN THE NASAL SECRETIONS

and not upon the bacterial content of the secretions   Generally, in the macrophage epithelial cell type of response occasional lymphocytes and eosinophiles were present   Red blood cells were encountered in the majority of specimens but a grossly bloody specimen was rarely encountered

This type of response seldom lasted more than forty eight hours By the beginning of the third day of the cold the polymorphonuclear leukocytes were abundant and by the fourth day their percentage out-

numbered that of all of the other types of cells   Twenty-three of the ninety-eight specimens of nasal secretions showed this type of response at the first examination

In the second type of response the percentage of polymorphonuclear leukocytes was always elevated from the first and earliest possible examination   These cells remained predominant throughout the entire course of the cold   Thirty-three of the ninety-eight specimens of nasal
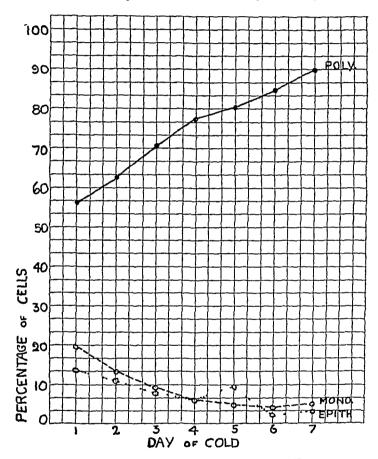
CHART IV   THE AVERAGE PREDOMINANT CELLULAR RESPONSE AS DETER-
MINED IN A STUDY OF THE NASAL SECRETIONS IN 98 COLDS

secretions showed 70 per cent or more polymorphonuclear leukocytes at the first examination   Thirty-nine specimens fell in between these two types of response in that the percentage of polymorphonuclears ranged between 49 and 70 in the initial specimens

There were three interesting examples of a variation from the general types of cellular responses seen in the nasal secretions in colds   This variation occurred in individuals who gave definite histories of having repeated attacks of pollen hay fever during the past years   In these

individuals there was a marked outpouring of eosinophilic leukocytes into the nasal secretions, which persisted throughout the early stages of the cold   The eosinophiles replaced the polymorphonuclear leukocytes to a large extent without disturbing the macrophage epithelial cell formula In each instance in which this was encountered the differential white blood cell count was well within normal limits

It has long been known that in individuals who suffer from hay fever, the nasal submucosal tissues are heavily infiltrated with eosinophiles and that this infiltration persists during the intervals when the acute allergic process has abated   Hence it is not surprising that these indi viduals should react to an acute infection of the nasal mucous membranes with an eosinophilic response in their nasal secretions

In our control group of individuals suffering from acute hay fever we invariably found a high percentage of eosinophile leukocytes in the nasal secretions   However, it was interesting to note that macrophages and epithelial cells were present in these secretions in a percentage that is commonly found in colds

Many of the air dried smears of the nasal secretions were stained with eosin methylene blue and the cells were searched for the presence of intranuclear inclusion bodies   In several instances homogeneous aci dophilic bodies were seen in the cytoplasm of columnar epithelial cells in the secretions from early colds   These closely resembled inclusion bodies but due to the lack of control studies upon normal epithelial cells we feel that the assumption that these acidophilic bodies are inclusion bodies would be unwarranted at the present time

DISCUSSION

It is our opinion that in general our findings confirm the observations of Hilding (2)   Undoubtedly the first changes taking place in the nasal mucous membranes during a cold must be an intercellular edema with congestion and an infiltration of macrophages or polymorphonuclear leukocytes   The epithelial cells become swollen and soon detach them selves from the submucosa and are found free in the nasal secretions The infiltrating macrophages and polymorphonuclear leukocytes either actively wander into the secretion or are passively swept out of the tissue spaces by the edema fluid

Within forty-eight to seventy-two hours the polymorphonuclear cells become the predominant cell in the nasal secretion and the secretion tends to change from a watery type to a mucopurulent type of discharge, and remains so throughout the remainder of the infection until the stage of crusting is over and the nasal mucous membrane has regenerated   It is well known that a monocytic tissue response is characteristic in certain virus diseases and we feel that the outpouring of monocytes seen in the nasal secretions in early colds may represent a response to the filterable agent of   common colds "

We realize that it is dangerous to argue by analogy, but nevertheless, we would like to call attention to the similarity of the changes taking place in the nasal mucous membranes in colds, to those taking place in the skin during vaccinia   There is first, the stage of engorgement of the nasal mucous membranes which resembles the formation of the vaccine papule   Then comes the tissue edema and destruction of the nasal mucous membrane with an outpouring of fluid containing monocytes which corresponds to the formation of the vesicle filled with fluid in which the mononuclear elements predominate   In the third stage of the cold the secretions have a high polymorphonuclear leukocyte content as does the fluid in the pustule of vaccinia, and finally in both diseases the last stage is characterized by crusting   Here the analogy ends because in colds the mucous membrane is restored while in vaccinia a scar generally results

CONCLUSIONS

There are two main types of cellular response found in the nasal secretions early in the course of colds   In the first type the macrophages and epithelial cells predominate and in the second the polymorphonuclear leukocytes predominate   Variations between these two types are commonly seen   By the third day of a cold the polymorphonuclear leukocytes become the chief cellular constituent of the nasal secretions   A third type of cellular response in colds in which eosinophiles predominate has been described   It is thought that these observations upon the cellular content of the nasal secretions in colds lend further evidence to the belief that a filterable agent is the cause of acute common colds

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MAGNESIUM METABOLISM IN HYPERPARATHYROIDISM

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In an earlier, rather extensive, publication in this journal on the "Functional Pathology of Hyperparathyroidism ' (1) no mention was made of magnesium metabolism Data have now been collected permitting a contribution to this phase of the subject The methods of study were the same as described in the previous paper (1) Magnesium determinations were made by the procedure outlined by Briggs (2)

The pharmacological relationship of calcium and magnesium ions and therefore the necessity for optimum concentrations of both in body fluids suggest a close association of the mechanisms regulating their level in the blood The fact that magnesium phosphate has solubility properties similar to calcium phosphate and the fairly constant amount of magnesium in bone hint at a specific biological relationship of their compounds

It would seem that the parathyroid hormone might have at least an indirect influence on magnesium metabolism The immediate effect of parathyroid hormone on serum magnesium has been studied in dogs by Scholtz (3) and by Greenburg and Mackey (4) Their investigations showed a slight increase in serum magnesium occurring early and before the rise in calcium By the time calcium had reached maximum values the serum magnesium had fallen towards normal Our experiments indicated that more prolonged administration of parathormone to dogs resulted in no striking alterations in serum magnesium (Table I) This was also true with rabbits in which parathormone is not very effective in raising the serum calcium

TABLE I

The effect of continuous parathormone administration on the serum magnesium of dogs and rabbits

	Serum magnesium	
	Before	After
	mgm per 100 cc	*mgm per 100 cc*
Dog No 1 parathormone, 50 to 300 units daily for 4 days	3 3	3 4
Dog No 2 parathormone 50 to 300 units daily for 4 days	3 0	5 5
Dog No 3 parathormone 50 to 100 units daily for 10 days	2 2	2 9
Dog No 4 parathormone 50 to 100 units daily for 10 days	2 1	2 9
Rabbit No 1 parathormone, 10 units daily for 10 days	2 6	1 9
Rabbit No 2 parathormone 10 units daily for 10 days	3 2	2 8
Rabbit No 3 parathormone 10 units daily for 10 days	2 8	2 9

In clinical *hypo*parathyroidism serum magnesium tends to be lower than the average for normal individuals   This is not a constant or striking phenomenon   Representative values in tetany following thyroidectomy are presented in Table II   For comparison with the *hypo*calcaemia of parathyroid tetany this table also includes observations on patients with *hyper*calcaemia in generalized neoplastic disease of bone, a condition which at least simulates hyperparathyroidism   These patients also showed low normal figures for serum magnesium

TABLE II

Serum magnesium, calcium and phosphorus of cases with hypocalcaemia and cases with hypercalcaemia

Cases with hypoparathyroidism following thyroidectomy				Cases with hypercalcaemia associated with generalized neoplastic disease of bone			
	Serum					Serum	
	Mg	Ca	P		Mg	Ca	P
	mgm per 100 cc	*mgm per 100 cc*	*mgm per 100 cc*		*mgm per 100 cc*	*mgm per 100 cc*	*mgm per 100 cc*
Case 11317, Tetany	2 1	8 2	4 4	*Case 22810*, Metastatic	1 9	14 9	2 5
	2 6	7 9	4 7	Hypernephroma	1 9	15 1	2 7
Case 22828, Tetany	1 7	7 6	5 5	*Case C S*, Multiple	2 4	13 5	6 8
	1 7	8 8	3 7	Myeloma	1 8	18 1	6 2
Case 23523, Tetany	1 7	7 8	7 7	*Case 27621*, Multiple	2 7	16 5	4 4
	1 8	5 9	5 9	Myeloma	2 0	16 9	4 9
					1 8	16 8	4 0

In *hyper*parathyroidism one finds evidence indicating more clearly that the parathyroid glands have no direct influence on the level of magnesium in the blood   This is illustrated in the studies of a typical case which will later be reported in detail by Olch   The data are recorded in Table III

Case 26253   This patient, a woman forty years old, had always considered herself healthy   She was raised in the city   Her food habits appeared to have been quite normal   Diphtheria in childhood was the only serious infectious disease she remembered   She was married when seventeen years old   Her only pregnancy was at eighteen years and was apparently quite normal and was followed by an uneventful puerperium   About this time dental caries began to develop and careful cooperation with her dentist failed to save her teeth   Fillings continued to fall out and caries advanced until finally all her teeth were removed   Her weight was continuously around 125 pounds until her hospitalization

Six years before admission the patient fell on the floor from the level of a bed and broke a clavicle   Three years later she fell on the ice and broke her left humerus   Two years before admission she developed dull pain above her

TABLE III

Indicating the influence of variations in parathyroid activity on serum magnesium

Case 20253 Hyperparathyroidism A parathyroid adenoma removed January 8 1931	Serum			Date
	Mg	Ca	P	
	mgm per 100 cc.	*mgm per 100 cc.*	*mgm per 100 cc*	
Before operation	1 6	16 2	1 9	Nov 4, 1930
Before operation	1 7	14 0	2 3	Jan 8 1931
One day after removing parathyroid tumor	1 7	9 8	1 7	Jan 9 1931
Four days after removing parathyroid tumor	1 7	8 3	1 7	Jan 12, 1931
Seven days after removing parathyroid tumor	1 8	8 9	2 4	Jan 15 1931
Eleven days after removing parathyroid tumor	1 9	9 6	2 1	Jan 19 1931
Eighteen days after removing parathyroid tumor	2 5	9 5	2 6	Jan 26 1931
Three months after removing parathyroid tumor	2 3	10 7	2 4	Apr 2 1931
One year after removing parathyroid tumor	2 5	10 2	3 6	Dec 18, 1931
Case 35690 Parathormone administration to a young woman normal except for otosclerosis				
Before parathormone	2 2	10 1	3 7	Sept 20, 1932
After 100 units parathormone in 24 hours	2 6	12 5	2 9	Sept 22 1932
After 160 units parathormone in 48 hours	2 7	12 5	2 9	Sept 23, 1932
After 400 units parathormone in 5 days	2 7	13 4	1 5	Sept 26, 1932
After 580 units parathormone in 7 days	2 6	12 8	2 3	Sept 28 1932
36 hours after stopping parathormone	2 5	10 9	2 5	Sept 30 1932
Four days after stopping parathormone	2 5	10 3	3 8	Oct 3 1932
Seven days after stopping parathormone	2 6	10 5	3 3	Oct 6 1932

left knee   This was thought to be rheumatism but, when it did not improve after months an x ray was taken   Her physician told her there was a lack of calcium in this area and that if she ever broke it healing might not take place   The pain gradually improved

The patient then felt perfectly well until June 25 1930, when she stumbled over a hedge and fell on a sidewalk and broke her left femur and right humerus X ray examinations showed these to be pathological fractures through multi locular cystic areas in the bone   Further x ray studies showed similar areas in other bones especially in the pelvis and upper dorsal spine   X ray also showed shadows in both kidney regions typical of bilateral nephrolithiasis

Physical examination disclosed little for comment except moderate emaci ation an upper dorsal kyphosis, hyperactive deep reflexes and a systolic blood pressure of 180 and a diastolic of 100   No tumor in the thyroid region was evident   Blood examinations showed high serum calcium, low serum phos phate, a slight anemia normal nonprotein nitrogen and negative Wassermann and Kahn reactions   The basal metabolic rate was − 4 per cent   Phenol-sulphonephthalein excretion was 70 per cent

On October 30 1930 surgical exploration of the thyroid region by Dr I Y Olch disclosed a small tumor, about 1 5 by 2 cm in size in the left lobe After this was removed the serum calcium did not fall and microscopic study showed it to be a foetal adenoma of the thyroid   On January 8, 1931, the neck was explored again and a tumor 1 5 by 4 cm in size was found lying in a

crevice between the oesophagus and spine and attached by a pedicle to the upper pole of the left lobe of the thyroid   After removing this tumor the serum calcium fell to below normal and the patient experienced moderately distressing symptoms of tetany for about ten days

Striking improvement in this patient's skeletal abnormalities, as evidenced by x-ray examinations, has followed operation   The improvement in her general condition has been less marked   The bilateral nephrolithiasis opposes a satisfactory course   Although there are no local symptoms, hypertension increases steadily and eventual kidney insufficiency is feared   Her general condition will not permit removal of the stones

While in the hyperparathyroid state the serum magnesium varied from 1 6 to 2 0 mgm per 100 cc   These figures are low but within the range of normal variations   Following the removal of the parathyroid adenoma the serum calcium fell typically to values below normal, but this change in state was not attended by any alteration in the level of serum magnesium   During the three weeks following operation magnesium varied from 1 8 to 2 0   Three months later when the calcium was 10 7 mgm and phosphorus 2 4 mgm the serum magnesium was 2 3 mgm per 100 cc   A year after operation the figures were about the same except that the serum phosphorus had risen to normal

Studies of magnesium, calcium and phosphorus metabolism of this patient, with clinical hyperparathyroidism, are recorded in Table IV Period 9, the first included in this table, was preceded by eight similar periods in which only the calcium and phosphorus were determined   As in period 9, they showed the typical negative balance of these elements A definite tendency to lose magnesium in hyperparathyroidism is indicated by the data in this table   A significant relationship to the hyperparathyroid state is emphasized by the change in the magnesium balance which followed the extirpation of a parathyroid adenoma   As the patient was transformed to relative hypoparathyroidism and the serum calcium fell to below normal, the loss of magnesium shifted to a distinctly positive balance   Magnesium was retained as large amounts of calcium and phosphorus were stored   One year after operation the patient was studied for one period on the same diet she was following at home   This contained large amounts of magnesium, calcium and phosphorus, chiefly from milk   The retention of the three elements was still quite marked

These modifications of magnesium excretion may be secondary to an effect of the parathyroid hormone on calcium or phosphorus of bone It is interesting to compare the ratios of the balances of magnesium, calcium and phosphorus with the proportions of these substances in bone Considering calcium as 100, one may calculate the proportions shown in Table V

It is apparent that a loss of calcium is accompanied by a loss of magnesium and phosphate and that a retention of calcium is accompanied by a retention of the other two elements as might be expected from the

TABLE IV

Magnesium calcium and phosphorus metabolism of patient 26253 with hyperparathyroidism and the influence of removing the parathyroid adenoma. Also metabolism studies of patient with generalized neoplastic disease of bone and hypercalcaemia. Corresponding serum magnesium calcium and phosphorus figures are recorded in Tables II and III

Period	4 day periods (1930-31)	Magnesium				Calcium				Phosphorus			
		Intake	Output	Urine	Stool	Intake	Output	Urine	Stool	Intake	Output	Urine	Stool
		grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams
	Case 26253												
9	November 4 to 7	1 128	1 444	0 539	0 905	14 41	22 51	2 24	20 27	7 00	8 01	5 02	2 99
12*	November 28 to December 1	1 065	1 038	0 634	0 404	11 76	7 04	1 79	5 25	7 02	7 86	5 65	2 21
13*	December 2 to 5	0 993	1 220	0 106	1 115	9 83	13 21	0 73	12 48	7 20	7 76	4 45	3 31
14*	December 6 to 9	1 044	1 136	0 365	0 771	10 29	10 17	0 86	9 31	7 38	6 95	4 88	2 07
15*	December 10 to 13	0 986	1 009	0 157	0 852	11 42	13 36	0 79	12 58	6 39	5 92	3 66	2 26
20	January 13 to 16	0 808	0 679	0 137	0 542	14 00	8 95	0 79	8 86	3 24	1 21	0 20	1 01
21	January 17 to 20	0 883	0 912	0 196	0 716	12 11	10 47	0 04	10 43	3 40	2 02	0 76	1 26
22	January 25 to 28	0 846	0 632	0 076	0 556	12 58	7 25	0 08	7 17	3 20	2 28	1 21	1 07
23	January 29 to February 1	0 827	0 671	0 066	0 605	11 88	3 88	0 09	3 79	3 69	1 74	1 09	0 65
24 25	December 17 to 20	2 414	1 854	0 554	1 300	15 82	7 51	0 07	7 44	11 25	6 87	4 86	2 01
	Case 27621												
1	January 28 to February 1	0 468	0 456	0 175	0 281	2 30	3 06	1 12	1 94	2 64	2 61	1 62	0 99
2	February 6 to 9	0 400	0 391	0 156	0 235	2 11	4 02	1 50	2 52	2 49	2 88	1 99	0 84

* In periods numbers 12, 13 and 14 irradiated ergosterol (Acterol 20 drops t i d) was administered in period number 15 cod liver oil Medication may have caused some tendency to retention of calcium and phosphorus

TABLE V

Ratios of magnesium and phosphorus to calcium

	Calcium	Magnesium	Phosphorus
	grams	grams	grams
Approximate proportions in bone*	100	2	50
Loss the month before operation	100	7 4	17 6
Retention the month after operation	100	2 3	31 4
Retention one year after operation	100	6 7	53 8

* Analysis of bone from another case of hyperparathyroidism gave the following figures Calcium 15 39 per cent, magnesium 0 23 per cent, and phosphorus 6 89 per cent   These would give ratios of 100 1 5   45

composition of bone   Significant variations from the ratios in bone do occur but further discussion of the data would not be profitable

A study of a patient with generalized neoplastic disease of bone and hypercalcaemia is included in Table IV (*Case 27621*)   This patient showed a markedly negative calcium balance and a slight loss of phosphorus   Nevertheless, she appeared to be in magnesium equilibrium

Magnesium metabolism was also studied in experimental hyperparathyroidism   A young woman 27 years old, who appeared normal except for moderate undernutrition and otosclerosis, was given 70 to 100 units of parathormone (Lilly) daily for eight days   Magnesium, calcium, phosphorus, nitrogen and sulphur balances were investigated, before, during, and subsequent to the hyperparathyroid state   The results of four day periods of study are summarized in Table V   During the preliminary observations she demonstrated an ability to retain all elements, probably because of an improved dietary regimen   During the eight days of parathormone administration there was a distinct increase in magnesium output, resulting in an evident negative balance   This was due entirely to a rise in urinary magnesium excretion   The serum magnesium rose slightly   When parathormone was discontinued there was a prompt storing of magnesium but no change in the level in the serum   In these observations the mobilization of calcium and phosphorus was quite typical   The excessive phosphorus excretion was greater than could be accounted for by calcium phosphate liberated and the magnitude of the increased protein metabolism   This surplus phosphorus was more than enough to explain the magnesium loss as magnesium phosphate released from the body   In the recovery period the retention of phosphorus could reasonably be accounted for by magnesium phosphate, calcium phosphates, and protein restored

The negative nitrogen and sulphur balance caused by parathormone may be related to the local inflammatory and slight general febrile reactions caused by the material injected rather than any specific effect of the hormone itself

TABLE VI

Magnesium, calcium, phosphorus, nitrogen and sulphur metabolism in four day periods of patient 35690, showing the effect of administering para-thormone for eight days. Parathormone was given through periods number 2 and 3. The amounts of parathormone and corresponding serum magnesium, calcium and phosphorus figures are recorded in Table III

4 day periods (1932)	Period	Magnesium				Calcium				Phosphorus				Nitrogen				Sulphur			
		Intake	Output	Urine	Stool	Intake	Output	Urine	Stool	Intake	Output	Urine	Stool	Intake	Output	Urine	Stool	Intake	Output	Urine	Stool
		grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams
September 17 to 20	1	0 859	0 821	0 406	0 415	2 74	2 08	0 63	1 45	3 78	3 08	1 96	1 12	35.0	32 4	29 4	3 0	2 76	2 29	2 04	0 25
September 21 to 24	2 *	0 835	0 871	0 503	0 368	2 68	3 11	1 41	1 70	3 54	5 45	3 25	2 20	30 7	35 1	32.5	2 6	2 19	2 55	2 31	0 24
September 25 to 28	3 *	0 923	1 184	0 816	0 368	2 84	3 48	1 78	1 70	3 66	5 01	2 81	2 20	35 1	36.6	34.0	2 6	2 85	2 70	2 46	0 24
September 29 to October 2	4	0 945	0 862	0 337	0 525	3 37	3 55	0 87	2 68	4 07	3 50	1 75	1 75	34 6	31 6	27 9	3 7	2 79	2 41	2 05	0 36
October 3 to 6	5	1 065	0 847	0 444	0 403	3 21	2 91	0 80	2 11	4 34	3 30	2 26	1 04	38 2	31 0	27 3	3 7	3 03	2 41	2 07	0 34

* By accident the stools of periods numbers 2 and 3 were mixed   For convenience of presentation one half of the total was assumed for each period

CONCLUSIONS

In the hyperparathyroid state there is a negative magnesium balance Individuals reverting from hyperparathyroidism to normal or the hypoparathyroid state store magnesium   Little evidence was obtained indicating the parathyroid glands have any direct effect on the level of magnesium in the blood   The influence of the parathyroid hormone on magnesium metabolism may be secondary to its action on calcium or phosphorus

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IMMUNITY IN DIABETES INFLUENCE OF DIABETES ON THE DEVELOPMENT OF ANTIBACTERIAL PROPERTIES IN THE BLOOD

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Notwithstanding the well known association of diabetes and infection we have little definite knowledge of the mechanism by which these two conditions affect each other   Since the days of Claude Bernard, clinicians have viewed with alarm either the appearance of infection during the course of diabetes or, perhaps with less apprehension, the occurrence of diabetes during or following an infection   In considering the coincidence of these disorders one might distinguish between the effect of each upon the other as different though essentially interdependent problems Various degrees of importance have been accorded to infection in the etiology of diabetes but there is general agreement regarding the harmful effect of infection when occurring during the course of diabetes

The resistance of the body to infection is attributed in part to the antigen amboceptor complement reactions of the blood   We have no knowledge regarding the influence of diabetes on these reactions nor on the formation of amboceptor or complement   The former may be present in the blood, as in natural immunity, or it may be formed following the inoculation of bacteria into the body, as in acquired immunity Complement appears to be always present and to react with either type of amboceptor   In this study, complement, natural amboceptor and acquired amboceptor, as factors in the antibacterial power of the blood, have been investigated in diabetic and non diabetic persons to determine whether this part of the protective mechanism of the blood is affected by diabetes

The complement of the serum was measured in hemolytic and bacteriolytic systems   The bactericidal power of the whole blood was chosen as the best method of determining the native amboceptor (1, 2)   The formation of agglutinins following inoculation with *B typhosus* vaccine was taken as the index of formation of acquired amboceptor

COMPLEMENT

Method   The hemolytic complement was measured in the sheep-rabbit hemolytic system   One half cc amounts of a 2 per cent suspension of washed sheep cells with an excess of amboceptor were taken with

increasing amounts of patients' serum and the total contents of each tube brought up to 3 cc with normal saline   The amount of serum necessary to provide an excess of amboceptor had been determined by preliminary titration   The tubes were then incubated at 37 5° C for 1 hour and the titre read as the smallest amount of serum that caused complete hemolysis   The sera were all fresh, the tests being done about 5 hours after the blood had been collected   On account of the variation in sheep cells, both diabetic and normal sera were set up in every series Whether the patients were taking insulin was noted

The patients were selected to represent wide variations of diabetes as regards severity, age, use of insulin and state of nutrition   A blood sugar was done on each blood taken

The bacteriolytic complement was measured by means of the bacteriolytic action of diabetic and non-diabetic sera on *B typhosus*   An excess of amboceptor in the form of human serum heated to 56° C for 30 minutes was added to increasing amounts of patients' sera and 0 1 cc of 24 hour broth culture of the organism   After 24 hours the tubes were cultured on blood agar plates and the titre taken as the lowest amount of serum which completely killed the *B typhosus*

Results   In all, 208 diabetic and 102 non-diabetic sera of patients without infection were examined   Of these, 191 diabetic and 97 non-diabetic were measured in the hemolytic system, while 17 diabetic and 5 non-diabetic individuals were used to determine the bacteriolytic complement   The complement titre of the serum in the non-diabetics varied from 0 020 cc to 0 140 cc with an average of 0 056 cc , while in the diabetics it varied from 0 030 cc to 0 120 cc with an average of 0 052 cc

In the measurement of bacteriolytic complement similar agreement was found   The titre of both diabetic and non-diabetic sera lay within the same range, namely, between 0 010 cc and 0 075 cc

Furthermore, diabetic patients with infection showed no change in the amount of their complement titre as compared with non-diabetics with infection   Nineteen of the former and 12 non-diabetics were set up In each group the titre varied between 0 020 cc and 0 080 cc   The averages for these groups were 0 040 cc and 0 052 cc respectively

It is evident from these studies that there is no significant variation from normal in the amount of complement present in the blood of patients with diabetes regardless of whether the blood sugar was high or low and whether or not they were taking insulin   The presence of infection did not appear to alter the complement content of the blood

BACTERICIDAL EXAMINATIONS

Since Nuttall in 1888 demonstrated the bactericidal action of whole blood, two methods of measuring the antibacterial titre have been

commonly selected   Todd (1) and others following Wright used de fibrinated blood, while Heist (2) used fresh coagulable blood   Both of these methods were employed in the present study with each patient and control   Five organisms were used throughout the entire series of determinations, namely, *B coli*, *Pneumococcus*, *Staphylococcus aureus*, *Streptococcus hemolyticus* and *Streptococcus viridans*   *B typhosus* and *B pyocyaneus* were also included in some experiments   Because of the length of time necessary to complete the work, cultures which had been growing as laboratory strains for some time were taken so that there would be little variation in their behavior   All of the organisms had been in artificial cultivation for at least a year   Transplants were made at frequent intervals on human blood agar and from these, broth cultures were made 18 hours before the test and incubated at 37 5° C   Blood sugar determinations were done on all blood specimens

The defibrinated blood was used according to the method of Todd as given by Ward (3)   Defibrination was done in 150 cc flasks containing glass beads and leukocyte counts were made in a number of cases before and after shaking in order to determine the loss in leukocytes   Dilutions of 1/10, 1/100, 1/1000, 1/10,000, 1/100,000 and 1/1,000,000 of the 18 hour broth cultures were made, and 0 1 cc of each dilution was placed in one of a series of small glass tubes   There was then added to each tube 0 5 cc of defibrinated blood   The tubes were sealed by heat, and placed in a rack attached to an electric motor so as to revolve at the rate of about 6 revolutions per minute   The entire apparatus was placed rotating in the incubator at 37 5° C   At the end of 24 hours the tubes were opened and cultures were made on human blood agar plates   These were incubated for 48 hours and examined for growth   In each test also, human blood agar plates were seeded with 0 1 cc of the 1/100,000 dilution of each organism and the colonies counted after incubation for 24 hours

In order to minimize the effect of variations in the number of bacteria present in the 18 hour broth cultures, each determination consisted of one normal and two diabetic persons, except in four instances when only one diabetic was used   As far as possible attempt was made to get a severe and a mild diabetic to compare with each non-diabetic control   Controls were members of the Institute Staff and medical students

The fresh coagulable blood was used according to the method of Heist (2)   Into sterile, cotton stoppered test tubes, 85 × 15 mm , were placed 0 1 cc of the same dilutions of the five organisms as already described, and these overlaid with 0 5 cc of freshly drawn blood   The tubes were gently shaken and allowed to stand until coagulated, when they were placed in the incubator at 37 5° C   After 24 hours, cultures were made and examined as in the method with defibrinated blood   Sugar determinations were done on all specimens of blood.

Results   The results of the bactericidal tests by the method using coagulable blood are given in Table I   In these are shown the number of diabetic patients whose blood killed the same number of organisms

Comparison of number of organisms killed by diabetic blood with number killed by non-diabetic blood   (Coagulable blood method)

	Diabetics whose blood killed at least 100 fold less bacteria than non diabetic control			Diabetics whose blood killed approximately the same number of bacteria as non-diabetic control			Diabetics whose blood killed at least 100 fold more bacteria than non-diabetic control			Total patients
Bacteria killed by diabetic blood / Bacteria killed by non diabetic blood	$\frac{1}{10\,000}$	$\frac{1}{1\,000}$	$\frac{1}{100}$	$\frac{1}{10}$	1	10	100	1 000	10 000	
B coli			2	17	19	8				46
Pneumococcus	2	3	8	12	14	4	1	1	1	46
Staphylococcus aureus		7	5	14	6	7	3	2		44
Streptococcus hemolyticus	3	4	6	10	14	6	1			44
Streptococcus viridans	3	6	3	16	14	2	1	1		46

as the normal control and the number whose blood killed less than or more than the control   This method of reporting the results of the tests was adopted because it seemed the simplest way in which the influence of variation in number of bacteria in the broth cultures could be eliminated   The diabetic bloods were compared only with the normal bloods which had been set up at the same time, with the same broth cultures   The difference between the largest and smallest numbers of bacteria in the series of cultures of any one organism was less than the 10-fold dilution which was used between successive tubes of the determinations, so that even if this variation were not considered, the error would be less than one dilution in the series of 6 tubes

It will be noted that the distribution of cases shows that diabetic patients in general are less able to kill the test bacteria than the non-diabetic controls   This seems to be especially true with the gram positive cocci against which phagocytosis has been shown to be especially effective It appears that the diabetic tends to be deficient in whatever antibacterial bodies are naturally present in the blood   From Table II it is evident that there is no significant correlation of distribution of the antibacterial power of the blood with variations in the blood sugar   There seems to be some factor in the diabetic, other than the blood sugar at the time of the determination, which influences the bactericidal effect

A comparison of results of the methods using defibrinated and coagulable blood gave essentially similar results   The same difference between diabetic and non-diabetic bloods could be recognized in both The method with coagulable blood generally showed a slightly higher bactericidal titre than did the method with defibrinated blood

TABLE II

Comparison of organisms killed by diabetic blood containing over 200 mgm and under 200 mgm of sugar to those killed by non diabetic blood

Bacteria killed by diabetic blood / Bacteria killed by non diabetic blood	$\frac{1}{10\,000}$	$\frac{1}{1\,000}$	$\frac{1}{100}$	$\frac{1}{10}$	1	10	100	1 000	10 000
B coli									
Under 200 mgm			1	8	12	6			
Over 200 mgm			1	9	7	2			
Pneumococcus									
Under 200 mgm	2	1	5	8	8	1	1	1	
Over 200 mgm		2	3	4	6	3			1
Staphylococcus aureus									
Under 200 mgm		5	3	11	5	4	1	2	
Over 200 mgm		2	2	3	1	3	2		
Streptococcus hemolyticus									
Under 200 mgm	2	3		8	9	4			
Over 200 mgm	1	1	6	2	5	2		1	
Streptococcus viridans									
Under 200 mgm	2	4	2	10	8		1		
Over 200 mgm	1	2	1	6	6	2		1	

Conclusions Diabetic whole blood, regardless of the level of its blood sugar, has in general a weaker bactericidal property than has non-diabetic whole blood as tested by standard methods

ANTIBODY FORMATION

In addition to the presence of complement and natural amboceptor, immunity against bacterial infection is influenced by the ability of the body to form antibodies for specific infections In order to compare the formation of an acquired antibody in diabetic and non-diabetic persons, the response to typhoid vaccine was investigated

Methods Three doses, 500 million, 1000 million and 1000 million respectively of *B typhosus* vaccine [1] were given subcutaneously at intervals of seven days to 41 diabetic and 39 non diabetic persons Record was kept of the occurrence of local or general reactions in both series reactions, if present, being noted as slight, moderate or severe

The vaccine used was freshly made in order to get the highest possible agglutinative titre in the blood It was given first to the diabetic patients and later to the non-diabetic controls There was probably very little difference in the potency of the vaccine in the two series as the last inoculation was given within 8 weeks of the time of making the vaccine

Blood for determination of agglutinative titre was taken 2 weeks and 10 weeks after the last dose The titre was determined by Dreyer s (4) macroscopic method, using typhoid antigen killed and preserved by

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[1] Furnished through the courtesy of Lederle Laboratories Inc for this work

formalin The same strain of *B typhosus* from which the vaccine was made was procured from the makers for the purpose of making the antigen The same antigen was used throughout the 8 weeks Known positive and negative sera were run with each determination, the positive serum regularly clumping the antigen at a dilution of 1/20,480

The non-diabetic controls consisted of the entering class of nurses at the Methodist Episcopal Hospital in Philadelphia The diabetic patients were ambulatory cases from the clinic, and varied in severity, though none were in such condition that giving the vaccine would be a dangerous procedure It was impractical to get the patients of the same young age group as the controls though most of the former were under 40 years Frequent blood and urine examinations were made, and on the basis of these, as well as of the clinical condition, the patients were divided into three groups, namely, those in good, fair and poor clinical condition The first were those who had always a normal blood sugar with no sugar in the urine and who felt generally well The second group comprised those who were in fair condition and who more or less successfully maintained their diets, their blood sugar levels lay between 140 and 200 mgm per 100 cc, and glycosuria was sometimes present The third were those who always had a blood sugar over 200 mgm·and sugar in most, if not all, of the urine specimens

TABLE III

Agglutinative titre of diabetic patients and non-diabetic controls

	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10240	1/20480
2 weeks after vaccine											
Non-diabetics				1			1	4	6	7	20
Diabetics			1	2	2	6	7	5	10	6	2
Good clinical condition							3	1	1	2	1
Fair clinical condition					1	2	2	3	6	4	
Poor clinical condition			1	2	1	4	2	1	3		1
10 weeks after vaccine											
Non-diabetics					1	2		4	11	19	1
Diabetics	1	4	2	6	6	15		4	3		
Good clinical condition			1	1	1	4		1			
Fair clinical condition		1		2	2	9		2	2		
Poor clinical condition	1	3	1	3	3	2		1	1		

Results In Table III are shown the results of the agglutination tests It is quite evident that there is a marked difference in the titre of the diabetic and non-diabetic series at the end of both 2 weeks and 10 weeks Fifty per cent of the non-diabetics agglutinated at a dilution

of 1/20,480 compared with 5 per cent of the diabetics, and 84 per cent of the non diabetics agglutinated at 1/5120 or above, compared with 44 per cent of the diabetics The persistence of titre of the serum for 8 weeks is much less in the diabetics than in the non diabetics At the end of this time 81 per cent of the non diabetics were found to agglutinate at a dilution of 1/5120 or more compared with 7 per cent of the diabetics The effect of the high blood sugar on the formation of antibodies does not appear to be definite, though there is perhaps a slightly less effective formation in the patients whose diabetic condition is described as poor

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SUMMARY

Complement in the blood of diabetic patients does not differ in amount from that in the blood of non-diabetics   This is true whether or not infection is present

The antibacterial power of the blood of diabetic patients as measured by standard methods tends to be less than that of non diabetics

Diabetic patients are less able than non diabetic controls to form agglutinins following their inoculation with typhoid vaccine

From these studies it appears that any deficiency in the antibacterial reactions of the blood of the diabetic comes rather from impairment of the amboceptor than from any lack of amount or activity of the complement   This is true of both the natural amboceptor as shown by the bactericidal test, and of the acquired amboceptor, as shown by formation of agglutinins

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A NOTE ON THE CALCULATION OF WATER EXCHANGE

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In a recent publication (1), Peters, Kydd, and Lavietes have "simplified" the method proposed by Newburgh and Johnston (2) for determining water exchange   Later publications from this laboratory (3), in which it was shown that the calculation of heat production directly from the insensible loss of weight is not accurate, have an important bearing on the suggestion of Peters, Kydd and Lavietes who propose to calculate heat production directly from the insensible loss of weight by means of their Equation 2

$$1\ 301\ (I\ L\ ) + 19\ 27 = Cal\ per\ hour,$$

where I L expresses insensible loss of weight per hour   This equation is derived from the data of Benedict and Root (4), who demonstrated the fact that the insensible loss of weight is roughly proportional to the heat production in the basal state

We have found that this relationship is not quantitative for long periods of varying activity during which food is ingested, because the R Q does not remain uniform, as it tends to in the basal state

The nature of the insensible loss of weight may be expressed algebraically as follows

$$I\ L\ = H\ O + CO_2 - O$$

It is obvious from the above equation that the only factor concerned in the dissipation of heat is the water that leaves the organism as vapor and carries with it 58 cal per gram   We have demonstrated that the heat lost in this way is 24 per cent of the total heat when certain precautions are in effect   Hence, the relationship is one between the vaporization of water and heat rather than between the insensible loss of weight and heat   While the water vapor is a component of the insensible loss of weight, there is no strictly quantitative relationship between the two, as may be seen from the following table

R Q	Water Vapor grams/~4 hrs	I L grams/24 hrs
1 00	978	1193
0 82	978	1054
0 707	978	940
	1151	

When the heat production calculated by the method proposed by us is compared with the "simplified" method advocated by Peters and his associates, the following values are obtained

R Q	According to Newburgh et al Cal/24 hours	According to Peters et al Cal/24 hours
1 00	2367	2116
0 82	2367	1833
0 707	2367	1403

Therefore the Equation 2 of Peters, Kydd and Lavietes entails a serious error which limits the value of the subsequent equations that depend upon the validity of this one

We agree with Peters, Kydd and Lavietes that preformed water should not be included in the water balance if one follows the standard practice in regard to balances   However, consideration of preformed water, as employed by Newburgh and Johnston, gives information that could not otherwise be obtained   They desired to secure a precise statement of the expected change in body weight in response to any given diet   It seemed proper to assume that, in the adult subject, there is an optimum value for the total volume of the circulating fluids   The water of the body is either part of this circulating fluid or of the protoplasm   It is generally recognized that, in the absence of disease, the per cent of water in the protoplasm is constant   Hence, any change in body weight that was not explained by change in the mass of protoplasm (i e solids plus their associated water), would be attributable to change in the volume of the circulating fluids   This latter phenomenon would then be a departure from the theoretical optimum   To evaluate this response, it was necessary to know how much water was to be apportioned to the gain or loss of protoplasmic solids   This increment of water was accordingly added algebraically to the sources of water, and was called "Preformed Water "   Newburgh and Johnston were not concerned with the question whether such water is "free" or "bound "

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THE RATE OF EXCRETION OF URINE IN SUBJECTS WITH DIFFERENT AMOUNTS OF RENAL TISSUE [1]

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In the vast literature on renal function, there is to be found only one method proposed as an indirect means of measuring the mass of functioning kidney tissue, namely, the Addis Ratio (1) $\left(\dfrac{\text{Urea in one hour's urine}}{\text{Urea in 100 cc of blood}} \right.$, under certain special conditions of diuresis and high blood urea concentration) That this method is an accurate index of functioning renal tissue has been shown by several considerations, summarized by MacKay (2) In each of three species (rat, rabbit, and dog) the value of the Ratio bears a direct linear relationship to the actual weight of the kidneys In man, a similar relationship has been demonstrated between the Ratio and body surface, while body surface and kidney weight are in turn in straight line association

The purpose of this paper is to study in man the relation between the amount of functioning renal tissue (as measured by the Ratio) and the volume of urine per unit of time under conditions designed to induce "minimum" and "maximum" rates of excretion [2] MacKay and MacKay (3) have already demonstrated the relation between the blood urea concentration and the amount of functioning renal tissue (similarly measured)

LITERATURE

Under stringent conditions of (a) urea and water administration and (b) withdrawal of dietary fluids Addis (4) found in man a relationship between the amount of kidney tissue and the rates of urine excretion He suggested that the disturbing influence of extra renal factors on the excretion rates might be diminished by using a "volume quotient" (the quotient obtained by dividing the "maximum" volume per hour by the "minimum" volume per hour) with which to predict kidney mass

Experimentally, the amount of renal tissue has been altered by subtotal nephrectomy, ligation of renal artery branches, and ligation of

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[2] It must be emphasized that no true minimum or "maximum" rate of urine excretion is attained These terms are used throughout this paper for brevity, and signify that the conditions are such as to induce a slow or rapid rate

kidney poles   Bradford (5) found in dogs a "considerable and practically permanent increase in the amount of urine passed" after about two-thirds of the total kidney mass had been excised, after the excision of three-fourths of the kidney mass, the polyuria was more marked   Mark (6), also in dogs, found that there was a reduction in the "maximum" rate of urine excretion when only small amounts of kidney remained, while the "minimum" rate of excretion increased   Chanutin and Ferris (7) observed the rate of urine excretion when water intake was restricted in rats after kidney pole ligation   Table 1 shows the mean values derived from their protocols

TABLE 1

"Minimum" rate of urine excretion in rats from which portions of the kidneys had been removed by pole ligation   (After Chanutin and Ferris (7))

Kidney weight per cent of normal	"Concentration test"	
	Cc per 24 hours	Specific gravity
100 0	2 4	1 058
75 6	10 8	1 024
66 6	15 0	1 017
31 2	15 6	1 014

As yet, no data have appeared showing in statistical terms how the rate of urine excretion is related to the mass of kidney tissue   Obviously, it would be very useful clinically if one could predict renal mass merely by measuring the volume of urine excreted in a given time   It was to determine whether this could be done that the present study was undertaken

METHODS

Five hundred and twenty-four sets of observations, collected in this laboratory during the past 10 years, were analysed by standard statistical methods (8, 9)   In each set the data included the (a) Addis Ratio as per cent of normal, (b) maximum rate of urine excretion as induced by water and urea administration, and (c) minimum rate of urine excretion as induced by withdrawing liquids from the diet

Subjects   The subjects were, for the most part, ambulatory patients with Bright's disease of varying type, severity, and duration   (Some were normal as judged by the Ratio, urinary sediment, and absence of proteinuria)   Age varied from 9 to 63 years   Body surface varied from 0 83 to 2 50 square meters, with a mean of 1 72 sq m   The majority were males   Many subjects were studied more than once

Diet   The diet varied with the type of the disease being treated Most subjects were taking a diet low in salt and adequate in protein and calories

Edema   A few subjects were seen when edematous and again in edema-free periods   All observations were included in the analysis

Conditions   Each subject received a copy of the following printed directions

DIRECTIONS

1 After breakfast take no fluids of any sort until next morning

This means no coffee, tea soup milk, etc., as well as no water

2 At 9 00 P M that evening void urine and throw it away

Do not void for some hours before 9 00 P M so that you may have no difficulty in emptying the bladder completely

After 9 00 P M if possible do not void urine again until next morning, but if it is necessary pass the urine directly into the special bottle which is provided

3 At 6 00 A M next morning pass urine directly into the special bottle

4 At 6 02 A M i e immediately after collecting the night urine in the special bottle, drink a large glass of water in which the urea in the box has been dissolved   Then during the next half hour slowly drink three other large glasses of water   The water may be taken hot if desired

5 At 7 A M void and throw away urine   Drink two glasses of water slowly

6 At 8 A M void and throw away urine   Drink two glasses of water slowly

7 At 8 50 A M be in the laboratory

8 Take no breakfast

After the subject came to the laboratory, his urine was collected and measured for three consecutive hours and blood specimens were withdrawn at the middle of each collection period   The rate of urea excretion (mgm in one hour's urine) and the concentration of urea in the blood (mgm in 100 cc) were determined, from this data the Ratio was calculated and expressed as per cent of normal for the subject's body surface (1)

Minimum volume   The volume of urine excreted during the restriction of dietary fluids was divided by the elapsed time to obtain the rate of excretion as cubic centimeters per hour   This rate was adjusted to the mean body surface of the group (1 72 sq m) before statistical analysis

Specific gravity of the "concentration test "   The specific gravity of the urine obtained during restriction of fluids was measured by a hydrometer at room temperature   No correction was made for proteinuria

Maximum volume   The maximum rate of excretion of urine in any one of the three hour periods during the execution of the Ratio was selected and expressed as cubic centimeters per hour   Before statistical analysis, this rate was corrected to a body surface of 1 72 sq m   The specific gravity of this urine was not recorded

Volume quotient   This variable $\frac{\text{maximum volume}}{\text{minimum volume}}$ was calculated in each of the 524 sets of observations, there was no need to correct it for surface area

Statistical terms   A few terms should be explained briefly for those not familiar with statistical methods (8, 9)

a *Correlation coefficient* (*r*) is a measure of the correlation of two variables when the means of the arrays fall upon a straight line, within the errors of sampling   (An array is a row or a column of a correlation table)

b *Correlation ratio* (η) is a measure of the correlation of two variables without regard to the linearity of the means of the arrays   The value of η given here is corrected in each case to allow for the influence of the number of the arrays (18)   When ζ/P E $_r$ is greater than 3, η is significantly greater than *r*, and may be used in place of *r*   ($\zeta = \eta^2 - r^2$)

c *Standard deviation* (σ) is a measure of dispersion or variation

d *Regression lines* are lines formed by plotting the means of the arrays   In this study curves have been fitted to them, when possible, by the method of least-squares   In every correlation of two variables, there are two regression lines (one the means of the rows, the other the means of the columns)   when they are at right angles, correlation is nil, when they coincide, correlation is unity   It is important to bear in mind that the regression of *x* on *y* allows *x* to be predicted when *y* is known, the regression of *y* on *x* allows *y* to be predicted when *x* is known

RESULTS

The results of the statistical analyses are presented in Table 2, Correlation Tables 3–6, Regression Tables 7–10, and in Figures 1–5

DISCUSSION

General considerations   The regressions were curvilinear (ζ/P E $_r > 3$) in the correlations of the Ratio with minimum volume, specific gravity of the concentration test, and volume quotient   The only linear regression (ζ/P E $_r < 3$) was found in the case of the maximum volume, which also showed the highest correlation with the Ratio

On theoretical grounds one expects that, of two tests of function having the same correlation ratios (η) with the mass of functioning kidney tissue, the test which has the more linear regression with renal weight would be the better test clinically   Thus, the correlation ratio (η) of blood urea concentration with the Addis Ratio is $-$ 0 90 (ζ/P E $_r$ = 4 3), but a study of the scatter diagram drawn by the MacKays (3), from which η was calculated, shows that the mass of kidney tissue may decrease to as little as 50 per cent of normal before the blood urea changes appreciably in concentration   Despite the high correlation ratio, the fact that the curve of the plotted observations is far from linear signifies that the blood urea concentration is an insensitive test of kidney mass   The MacKays' curve, in fact, approaches the shape of a rectangular hyperbola, any variable which actually formed the latter curve when plotted against kidney weight would plainly be useless as a clinical test, even though the correlation approached unity

Therefore, the maximum rate of urine excretion, because it happens to have a linear regression and a comparatively high correlation with the Ratio, is the best measure of kidney mass of those variables studied in this paper

TABLE 2

Statistical characteristics of the Addis Ratio (per cent of normal) with minimum volume specific gravity of concentration test maximum volume and volume quotient The Addis Ratio is denoted by x in each instance the other variable by y

Characteristic	Minimum volume	Specific gravity concentration test	Maximum volume	Volume quotient
r	$-0\,354 \pm 0\,026$	$+0\,530 \pm 0\,021$	$+0\,697 \pm 0\,015$	$+0\,568 \pm 0\,020$
η_{xy} $\mathfrak{x}/PE_{\mathfrak{x}}$	$-0\,351$	$+0\,577$ 4 00	$+0\,710$ 2 39	$+0\,683$ 7 44
η_y $\mathfrak{x}/PE_{\mathfrak{x}}$	$-0\,399$ 3 23	$+0\,554$ 2 77	$+0\,705$ 1 84	
M_x	$62\,53 \pm 0\,77$	$62\,53 \pm 0\,77$	$62\,53 \pm 0\,77$	$62\,53 \pm 0\,77$
M_y	$31\,88 \pm 0\,54$	$1\,0255 \pm 0\,0002$	$437\,3 \pm 5\,73$	$17\,95 \pm 0\,36$
σ_x	$26\,01 \pm 0\,52$	$26\,01 \pm 0\,52$	$26\,01 \pm 0\,52$	$26\,01 \pm 0\,52$
σ_y	$18\,22 \pm 0\,38$	$0\,0072 \pm 0\,0001$	$194\,4 \pm 4\,05$	$12\,29 \pm 0\,25$
N	524	520	524	524

Regression of Addis Ratio (per cent of normal) on minimum volume
$$x = 78\,628 - 0\,505y$$
Regression of Addis Ratio (per cent of normal) on specific gravity of concentration test
$$x = 1909y - 1896$$
Regression of Addis Ratio (per cent of normal) on maximum volume
$$x = 0\,0933y + 21\,728$$
Regression of Addis Ratio (per cent of normal) on volume quotient
$$x = 75\,3 + 0\,1243y - 60\,89e^{-0\,1207y}$$

Prediction of the amount of kidney tissue Figure 1 shows in line-chart form the most probable predictions of the amount of functioning kidney tissue (expressed as per cent of the normal Ratio) from a given rate of urine excretion under the conditions outlined above Each line in the chart represents the calculated regression line of the Ratio on the appropriate variable

In 50 cases, the Ratio was predicted (by means of Figure 1) from the maximum volume, minimum volume, specific gravity of the concentration test, and volume quotient, the predictions were compared with the actual Ratios and the root mean square deviations (8) of the errors calculated The deviations were 29 8 for the minimum volume, 19 9 for the maximum volume, and 22 1 for both the volume quotient and the

TABLE 3

Correlation table   Addis Ratio (per cent of normal) and minimum volume (cubic centimeters/hour/1 72 sq m)

Addis Ratio—per cent of normal

Minimum volume—cc/hour	0-4.9	5-9.9	10-14.9	15-19.9	20-24.9	25-29.9	30-34.9	35-39.9	40-44.9	45-49.9	50-54.9	55-59.9	60-64.9	65-69.9	70-74.9	75-79.9	80-84.9	85-89.9	90-94.9	95-99.9	100-104.9	105-109.9	110-114.9	115-119.9	Totals
140-149.9			1																						1
130-139.9						1																			1
120-129.9																									
110-119.9					1																				1
100-109.9																									
90-99.9		1	1	1	1	1																			5
80-89.9																									
70-79.9	1	1	1					1		1		1													12
60-69.9	1	1	2			1	2	1	2	1	1	2													20
50-59.9	1	3	3	1	1	1	4	3	2	3	2	3	1	2	2	1	1	3	2		2				35
40-49.9		1	3	2	2	3	4	3	2	2	3	2	1	3	1	1	1	4	3	1	1	1			48
30-39.9		1	2	3	2	9	11	5	6	3	8	9	8	6	5	4	7	5	4	4	1	2	1		101
20-29.9			1	3	1	4	9	3	4	11	10	14	10	14	7	20	15	14	11	7	7	4	2	1	161
10-19.9				1	1	2	4	1	1	5	5	14	11	11	7	13	10	10	11	5	4	4	2	1	129
0-9.9													2	1	1	6									10
Totals	3	8	14	11	9	22	34	17	17	26	29	45	33	37	23	45	34	36	31	17	15	11	5	2	524

TABLE 4

Correlation table   Addis Ratio (per cent of normal) and specific gravity of concentration test

Specific gravity—concentration test	Addis Ratio—per cent of normal																								Total
	0–4.9	5–9.9	10–14.9	15–19.9	20–24.9	25–29.9	30–34.9	35–39.9	40–44.9	45–49.9	50–54.9	55–59.9	60–64.9	65–69.9	70–74.9	75–79.9	80–84.9	85–89.9	90–94.9	95–99.9	100–104.9	105–109.9	110–114.9	115–119.9	
1 046–1 047											1														1
1 044–1 045											1	1													1
1 042–1 043												1	3												2
1 040–1 041												1	1	4	1	1	1								10
1 038–1 039									2	1			1	6		1	3	2							13
1 036–1 037									2	3	3	2	4	4	1	2	4	3	2	1		1			33
1 034–1 035						1	2	1	4	6	3	3	2	3	3	5	7	4	3	4	2	1	1		44
1 032–1 033						1	2	5	1	4	4	5	4	7	4	6	3	7	5	4	2	2	1		56
1 030–1 031						1	1	1	2	3	5	7	8	5	3	6	4	5	5	4	3	1	2		57
1 028–1 029						4	3	3	1	3	6	7	4	2	4	8	4	5	5	1	2	2	1		56
1 026–1 027					2	2	4	1	1	3	3	5	3	4	3	5	1	4	2	4	2				52
1 024–1 025					1	7	5	3	1	2	1	4	1	1	2	1	4	3	3	1	1			1	46
1 022–1 023					5	2	4	1	1	1		2			1	2	1	1	2		1	1		1	35
1 020–1 021				1	1	2	5	3	1			3	1			4			1						32
1 018–1 019				8		2	4	1	1			4													24
1 016–1 017			1	2	1	1	3		3	1		2	1												24
1 014–1 015	1	1	3		5	1	1					3													3
1 012–1 013			3																						1
1 010–1 011		2	3																						
1 008–1 009		2	2																						
1 006–1 007		3	2																						
1 004–1 005	2																								
Totals	3	8	14	11	9	22	34	17	17	26	29	46	33	37	22	44	33	35	30	17	15	11	5	2	520

TABLE 5

Correlation table — Addis Ratio (per cent of normal) and maximum volume (cubic centimeters)/hour/1.72 sq m)

Maximum volume—cc/hour	0–4.9	5–9.9	10–14.9	15–19.9	20–24.9	25–29.9	30–34.9	35–39.9	40–44.9	45–49.9	50–54.9	55–59.9	60–64.9	65–69.9	70–74.9	75–79.9	80–84.9	85–89.9	90–94.9	95–99.9	100–104.9	105–109.9	110–114.9	115–119.9	Totals
960–999																						1			1
920–959																									1
880–919								1																	2
840–879															1				2		1	1			5
800–839			1																						1
760–799														1			1		1	1	1	1			12
720–759												1	1						1	2	3	2			20
680–719											1					3		3	3	1	1				18
640–679										1	1	1	1	1		1		3	5	2	2				23
600–639										3	1	1	1	2	2	4	5	3	2	2		1			28
560–599							2	1	1		4	2	3	3	2	4	2	3	2				1	1	36
520–559							1	1	3	1	1	3	4	3	3	6	3	2	2	3	4	1	1		34
480–519					1		3			3	2	3	4	5	3	5	6	3	3	2		1	1		40
440–479					3		3	4	2	3	6	10	3	5	3	3	2	4	3	1	2	2	1	1	44
400–439						4	2	1	1	3	4	6	6	2	1	3	2	6	1						40
360–399			3	5	1	5	4	2	3	5	1	5	4	3	1	5	5	2							44
320–359			4	3	2	4	5	2		2	1	5	4	5	4	4	3	3	3	2					31
280–319			3	2	1	5	5	2	1	1	1	2		2		1	1	1	3		1				19
240–279		1	1	1		3	2	1	2	1	1	2	2		2	2							1		28
200–239		1	1			1	4	2	2	1	2	1		4		2	1								25
160–199		1	1				1		1		1				1	1						1			23
120–159		2					1			2		2					1								18
80–119	1	3					1		1			1				1									16
40–79									1								1								10
0–39	2																								5
Totals	3	8	14	11	9	22	34	17	17	26	29	45	33	37	23	45	34	36	31	17	15	11	5	2	524

TABLE 6

Correlation table   Addis Ratio (per cent of normal) and volume quotient

Volume quotient	Addis Ratio—per cent of normal																								Totals
	0–4.9	5–9.9	10–14.9	15–19.9	20–24.9	25–29.9	30–34.9	35–39.9	40–44.9	45–49.9	50–54.9	55–59.9	60–64.9	65–69.9	70–74.9	75–79.9	80–84.9	85–89.9	90–94.9	95–99.9	100–104.9	105–109.9	110–114.9	115–119.9	Totals
69–71.9																			1						1
66–68.9																				1					1
63–65.9																					1				1
60–62.9																									0
57–59.9													1												1
54–56.9																			1	1	1				4
51–53.9													1		1	1									3
48–50.9										1							1				1				3
45–47.9										1						1			1						3
42–44.9									1			2			1	1		1							6
39–41.9													1	1	1	1	2	1	2				1	1	10
36–38.9										2		1	1			1	1	4	1	1					9
33–35.9			1				1		1	6	2	6	2	2		4	3	4	3			2		1	13
30–32.9			1						2	2	5	2	5	5	4	9	3	1	2	4	1		2	1	24
27–29.9						1	1		1	3	5	5	5	8	2	10	3	3	1	2	1	1			27
24–26.9						2	2		2	2	6	3	4	3	2	2	5	3	3	4	1	1	1		40
21–23.9				1	1	4	8	4	2	3	1	7	5	5		3	7	5	1	2	3	1			39
18–20.9			1	2	4	5	8	10	2	2	4	5	3	6	2	3	4	6	2	1	1	1	2		43
15–17.9			1	7	4	9	10	3	2	4	4	5	4	2	3	6	2	4	7	3	1	3			51
12–14.9			7		4	1	4		1	3	3	1	2	3	4	2	1		1		2	1	1		48
9–11.9		1							3	1	3			2		4	1		3	2	1				58
6–8.9		2																	4	2	2				57
3–5.9		5																	1	1	1	1			31
0–2.9	3																1								
Totals	3	8	14	11	9	22	34	17	17	26	29	45	33	37	23	45	34	36	31	17	15	11	5	2	524

FIG 1 LINE CHART FOR PREDICTING ADDIS RATIO FROM MINIMUM VOLUME (CC/HR/1 72 SQ M), MAXIMUM VOLUME (CC/HR/1 72 SQ M), SPECIFIC GRAVITY OF CONCENTRATION TEST, VOLUME QUOTIENT, AND BLOOD UREA (MGM/100 CC) (BLOOD UREA LINE CALCULATED FROM DATA OF MACKAY AND MACKAY (3))

specific gravity   These results mean roughly that the odds are 2 to 1 in favor of a prediction being within ± 30 per cent [3] of the true value when the minimum volume is used, and within ± 20 per cent when any of the other variables are used to estimate renal mass
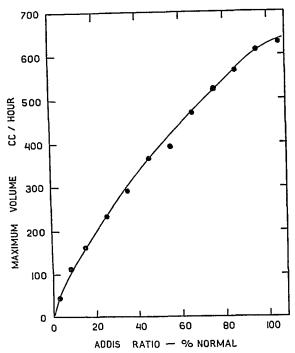
Fig 2   The Regression Line of Maximum Volume (Cubic Centimeters /Hour/1 72 Sq M) on the Addis Ratio (Per Cent of Normal)

Regression lines of the studied variables on the Addis Ratio   The regression line of the maximum rate of urine excretion on the Ratio is shown in Figure 2   The means fall quite smoothly along a parabola, which when extended meets the origin   The maximum rate begins to decrease almost as soon as kidney tissue becomes less than normal in amount   the slight lag in response may be an artefact due to the small

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[3] In per cent of the normal, not of the actual Ratio in any specific instance

number of observations at 105 per cent of normal, or may mean that the stimulus is insufficient to provoke a maximal renal response
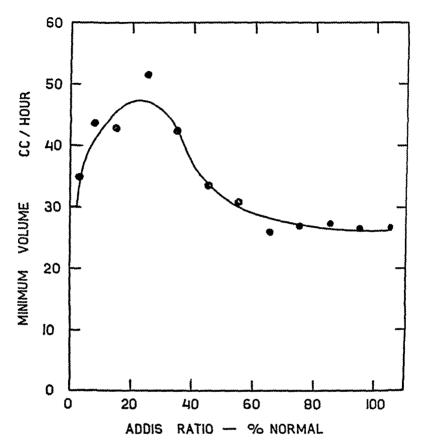
FIG 3   THE REGRESSION LINE OF MINIMUM VOLUME (CUBIC CENTIMETERS /HOUR/1 72 SQ M) ON THE ADDIS RATIO (PER CENT OF NORMAL)

The behavior of the minimum rate of excretion of urine is shown in Figure 3   The curve is not so smooth, and is of a more unusual shape The capacity of the kidney to produce urine at a slow rate is not involved until about 40 per cent of renal tissue is removed, a polyuria then follows, reaches its height when only 25 per cent of tissue remains, and declines rapidly toward anuria as the mass of kidney disappears

The regression line of specific gravity of the "concentration test" on the Ratio is given in Figure 4   As kidney substance is removed, the kidney is able to produce urine of high specific gravity until about 20 per cent of the tissue is gone, the urine's specific gravity then rapidly decreases at a rate which does not slacken until only 30 per cent of the original kidney weight remains

The volume quotient may be looked upon as a measure of the adaptability of the kidney to diametrically opposite stimuli (to excrete urine at a "maximum" and at a "minimum" rate)   Figure 5 shows that this
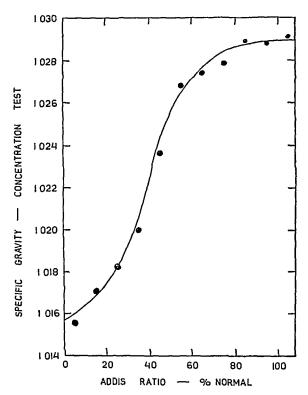
FIG 4   THE REGRESSION LINE OF THE SPECIFIC GRAVITY OF THE CONCEN
TRATION TEST ON THE ADDIS RATIO (PER CENT OF NORMAL)

adaptability is affected almost as soon as tissue is removed and decreases in a straight line until the kidney is able to excrete urine at only one rate regardless of the stimulus
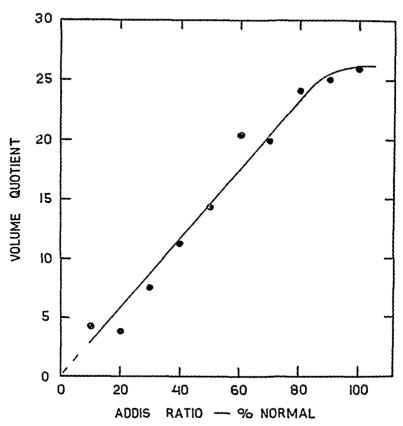
FIG 5   THE REGRESSION LINE OF THE VOLUME QUOTIENT ON THE ADDIS
RATIO (PER CENT OF NORMAL)

The data for the four related regression lines are given in Tables 7
to 10

TABLE 7

*Regression line of Addis Ratio (per cent of normal) on maximum volume
(cubic centimeters/hour/1 72 sq m)*

Maximum volume	Addis Ratio	Number of observations
40	23 5	15
120	31 2	34
200	39 9	48
280	47 1	47
360	57 0	75
440	69 7	84
520	74 4	74
600	73 9	64
680	84 8	41
760	88 5	42
		524

TABLE 8

Regression line of Addis Ratio (per cent of normal) on minimum volume (cubic centimeters/hour/1 72 sq m)

Minimum volume	Addis Ratio	Number of observations
5	53 5	10
15	72 7	129
25	69 1	161
35	57 8	101
45	50 3	48
55	53 6	35
65	41 7	20
75	42 0	12
85		0
95	38 5	5
105		0
115	22 5	1
125		0
135	27 5	1
145	12 5	1
		524

TABLE 9

Regression line of Addis Ratio (per cent of normal) on specific gravity of the concentration test

Specific gravity	Addis Ratio	Number of observations
1 0055	27 5	1
1 0095	50 8	3
1 0135	32 1	48
1 0175	40 6	60
1 0215	57 3	81
1 0255	64 4	108
1 0295	73 0	113
1 0335	79 9	77
1 0375	81 6	23
1 0415	63 8	4
1 0455	55 0	2
		520

TABLE 10

Regression line of Addis Ratio (per cent of normal) on volume quotient

Volume quotient	Addis Ratio	Number of observations
3	29 7	88
9	52 0	109
15	69 5	99
21	72 9	82
27	75 8	67
33	80 0	37
39	82 0	19
45	87 5	9
51	80 0	6
57	73 5	5
63		0
69	87 5	2
		524

SUMMARY AND CONCLUSIONS

A statistical analysis was attempted in correlating the Addis Ratio (as a measure of the amount of functioning renal tissue) with the rates of excretion of urine under specified conditions designed to induce minimum and maximum rates of excretion

Tests of function are discussed from the view-point of the shape of their regressions with the amount of functioning tissue which they are designed to measure The shape of the regression is at least as important as the correlation in determining the clinical value of the test

The odds are 2 to 1 that the Ratio may be predicted within a range of ± 20 per cent by means of urine excretion rates

The behavior of the rates of excretion of urine with different amounts of kidney tissue as measured by the Ratio is described No explanation for the shape of the curves is given

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SALT AND WATER IN THE TREATMENT OF DIABETIC ACIDOSIS

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In a previous paper (1) the nature and causes of the bicarbonate deficit and other serum electrolyte changes in diabetic acidosis were discussed from a theoretical standpoint, and disordered electrolyte patterns actually observed were analyzed in the light of this theoretical discussion It was pointed out that displacement of bicarbonate from combinations with base by ketone acids is a process which can be reversed completely with the contribution from outside sources of nothing except the ability to burn carbohydrate Base in combination with ketone acids may be considered potential bicarbonate On the other hand, the loss of base with ketone acids in the urine introduces an irreversible process, because base for the replacement of that sacrificed must come from external sources Such base deficiences may become quite extreme, but ordinarily they are small compared with the deficiences of base combined with chloride The actual extent of the body chloride losses is hardly evident from the concentrations of this ion found in the serum, because it is masked by contractions of the body fluids Such a contraction brought about by loss of water in excess of dissolved salts will increase the concentration of chloride more than it will that of bicarbonate, because the body fluids contain more chloride than bicarbonate In observed cases, the actual reduction of chloride concentration in the serum usually exceeded that of bicarbonate plus undetermined acids (which for convenience was called S and represents the actual plus potential bicarbonate mentioned above) If loss of body fluids is taken into consideration it is obvious that the actual loss of chloride must far exceed that indicated by the reduction in concentration The deficiency of base, of course, exceeds that of either chloride or S, because it represents the sum of the two base fractions In some instances, with S well preserved, the reduction of chloride concentration exceeds that of base This has been interpreted as evidence that the chloride ion, instead of the bicarbonate ion, has yielded base for the neutralization of ketones, a reaction which would seem to be neither useful nor chemically possible It was pointed out that if equal amounts of base and chloride were excreted and the body fluids were at the same time diminished by a disproportionately large elimination of water, the reduction in concentration of chloride would exceed that of

base, because contraction of fluid volume would increase base concentration more than chloride   It was suggested that this is a more likely explanation of excessive chloride deficits than the previous theory that the chloride ion is excreted with ammonia to provide base for the neutralization of ketones

The present paper is intended to analyze the clinical and therapeutic implications of these theories in the light of observations made in a series of patients during recovery from diabetic acidosis   The clinical material consists of twenty-five patients on whom studies of blood sugar together with total acid-base equilibrium of the serum were made at frequent intervals by the methods usually employed in this department (2)   In certain instances urine was analyzed for total base or chloride or both   The intakes of fluid, sodium, chloride and glucose were usually measured   Records of output are seldom so complete, because urine specimens and vomitus were frequently lost   Of these twenty-five patients, thirteen are discussed below   The remaining cases so closely resembled those reported that the inclusion of additional data does not seem warranted

In the discussion that follows it is assumed that insulin and carbohydrate were given to all cases with the intention, at least, of reestablishing glucose combustion and thereby eliminating ketosis as rapidly as possible   If this aim is realized, base previously combined with the ketone acids will be released to form bicarbonate   There can be no difference of opinion concerning the objective of therapy in this respect   Differences in the means of securing this objective will be discussed in another connection   The chief concern of the present study is the nature and quantity of fluids and salts which it is advisable to give   For the most part treatment actually employed has been the administration of large amounts of sodium chloride and fluids together with carbohydrate   When patients have been able to take these materials by mouth the sodium chloride has been given chiefly in the form of non-nutritive broth containing 1 per cent of this salt, the carbohydrates as sweetened drinks containing about 10 per cent of sugar   In addition, liberal amounts of water have been permitted   When oral feeding was impossible 0 9 per cent sodium chloride and 5 per cent glucose solutions have been given subcutaneously or intravenously, and occasionally stronger glucose solutions intravenously   The salt solution is intended to restore the volume of the body fluids and to remedy salt depletion   Glucose solution serves two purposes   1 It provides carbohydrate for combustion and replenishment of glycogen stores   2 It offers a medium for the parenteral administration of water without salt   Normal salt solution provides sodium in concentration approximately equivalent to that of total base in body fluids   In respiration and insensible perspiration there is a continuous wastage of fluid without any appreciable quantity of salt   Unless the kidneys, then, excrete base in concentration exceeding that of

body fluids, administration of normal saline alone would raise base in the body fluids to an abnormally high level   To obviate this difficulty, in addition to the saline, enough water should be given to provide for losses by extrarenal channels   Glucose solutions answer this purpose because the glucose can be oxidized, leaving only the water

The analysis of the data presented is complex not only because of the large number of variables with which it is necessary to deal, but also because the serum analyses are concerned with concentrations of sub stances and give no direct information as to total amounts of fluids and solutes in the body   In a preceding article the average compositions of body fluids in normal subjects and patients with diabetic acidosis were compared (1)   In Figure 1, diagrams A and B represent schematically
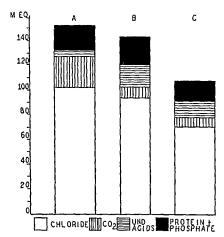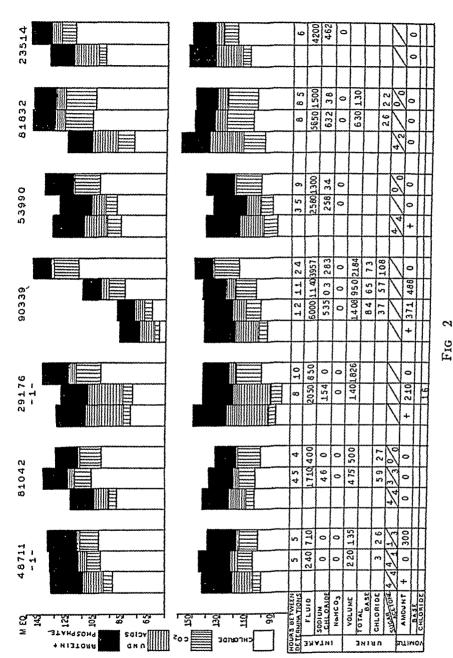
FIG 1   DIAGRAMMATIC REPRESENTATION OF THE CHIEF ELECTROLYTE AND WATER CHANGES THAT OCCUR IN DIABETIC ACIDOSIS

The width of columns B and C represents roughly the total body fluid volume compared to the normal A

the two conditions   As far as concentrations are concerned they are quite realistic because all values are derived from observed data   The 25 per cent reduction of body fluid in B represents little more than a guess, because the actual extent of fluid losses during the development of diabetic acidosis has not been established   Diagram C represents the electrolyte concentrations of B reduced by dilution to the original fluid volume found in A Thus C represents the actual salt losses found in diabetic acidosis   It emphasizes the necessity of having some measure

		48711 -1-	81042	29176 -1-	90339	53990	81832	23514
	HOURS BETWEEN DETERMINATIONS	5 5	45 4	8 10	12 11 24	3 5 9	8 5	6
INTAKE	FLUID	240 710	1710 400	2050 850	6000 1140 3957	2560 1300	565 1500	4200
	SODIUM CHLORIDE	0 0	46 0	154 0	535 03 283	258 34	632 38	462
	NaHCO₃	0 0	0 0	0 0	0 0 0	0 0	0 0	0
URINE	VOLUME	220 135	475 500	1401 826	1408 950 2184		630 130	
	TOTAL BASE				84 65 73			
	CHLORIDE	3 26	59 27		37 57 108		26 22	
	SUGAR	4 4	43 30			44	42	
	ACETONE	4 3	4 4 3 0				0 0	0
VOMITUS	AMOUNT	0 300	0 0	210 0	371 488 0		0 0	0
	BASE	+	0	+	+	44 +		
	CHLORIDE			16	0			0

FIG 2

FIGS 2 AND 3   ELECTROLYTE CHANGES DURING RECOVERY FROM DIABETIC ACIDOSIS

The lower diagrams represent the concentrations of electrolytes determined The upper diagrams represent these data recalculated as described in the text Total base is not shown but equals the total height of the acid column Volumes are expressed in cc, electrolytes in m Eq Sugar and acetone in the urine were determined by Benedict's qualitative method and the nitroprusside reaction respectively The figures 1 to 4 indicate roughly the intensity of the reactions The specimens of urine and vomitus reported in the first column in each case were produced before treatment was started The specimens reported in other columns were collected during the periods indicated

FIG 3

of dehydration in order that electrolyte concentrations determined after various forms of therapy may be adequately compared with the initial concentration   In a previous paper (3) the relation of the serum proteins to hemoconcentration has been discussed   It was pointed out that, except in the presence of shock, the fall of serum proteins from their initially high values probably is associated with, even if it is not exactly proportional to, restitution of body fluid   In the absence of a better and more exact method, the drop in the level of the serum proteins has been used in the succeeding discussion as one measure of body fluid change   Knowing the fluid and salt balances of an individual during a period of time, the changes that occur in the serum electrolyte concentrations afford another measure of body fluid change, if isotonicity of all the body fluids be assumed   It is only by correlating concentrations of electrolytes with changes in body fluid volume that the extent of the original depletion of the salts and their replacement during recovery become apparent

The analytical data obtained in thirteen of the cases investigated are presented in Figures 2 and 3   The bottom row of diagrams in these figures represents the serum electrolyte concentrations found   Total base is not shown, but equals the total height of the acid column in each case   The top row of diagrams presents the data recalculated to take into account water change   This was accomplished by using the changing serum protein concentrations as an index   For example, in Case 60404, the initial serum protein concentration was 18 9 m Eq and the final, 13 9 m Eq   Assuming that no protein was lost or gained, the serum volume must have increased 35 9 per cent   All the electrolyte concentrations found at the time of the first determination have been reduced 35 9 per cent in order to eliminate changes in concentration due to differences in the amount of water present   Thus the initial chloride concentration of 98 2 m Eq becomes 69 3 m Eq   Each determination has been compared to the final one in order that the approximate amounts of each ion added could be ascertained   The amount of salt in the intake and in the urine and vomitus are expressed in milliequivalents   Chloride was given in the form of sodium chloride, so that equal amounts of sodium and chloride were given

In analyzing the data, not only must the effect of changing body water content on the concentration of the electrolytes be evaluated, but also the results of vomiting and nausea must be ascertained   Unfortunately, vomiting, or at least nausea, is one of the most frequent events during the course of diabetic acidosis   Individuals who are vomiting are obviously losing from the body varying amounts of salt and fluid   They have in the intestinal tract a large volume of fluid which, although isotonic with the body fluids in general, may be of entirely different composition in regard to particular ions   Fluid placed in the gastro-in-

testinal tract eventually becomes isotonic This is true not only in individuals who are actually vomiting but also in those who are nauseated When, during recovery, the gastro intestinal symptoms diminish, this fluid is reabsorbed into the mass of body water These facts explain otherwise incomprehensible alterations in salt concentrations and fluid balances, observed during recovery, which are not related to the amount of salt and fluid immediately taken into the body or excreted Case 29176-1 furnishes a qualitative example of these changes In the first eight hours the patient received 2050 cc of fluid with 154 m Eq of sodium chloride Throughout this time he was vomiting or was nauseated The water content of the blood was apparently maintained, but base and chloride concentrations diminished, S remained constant In the next 10 hours his nausea disappeared Although he received only 850 cc of fluid with no salt during this period and passed 1820 cc of urine, there was about a 10 per cent blood dilution with a marked rise of serum chloride The concentration of base in the serum remained the same, but as the recalculated diagram shows, the total amount of base actually increased The most reasonable explanation of these changes seems to be that fluid was first poured out into the alimentary canal, and then reabsorbed This subject forms a part of a general discussion of water balance to be reported by Lavietes, Kydd and Peters (4)

In order to test the effect of varying therapy the individuals have been divided into four groups 1 The patients who were treated with insulin and carbohydrate alone 2 The patients who were given fluids in addition 3 The patients who received varying amounts of salt in addition to the therapy used in 1 and 2 4 The patients who received bicarbonate as well as salt, fluid, carbohydrate and insulin It has already been stated that the proportions of insulin and carbohydrate used will not be discussed in this paper

1 Treatment without salt and fluids

Case 48711-1 was given enough fluid (as carbohydrate drinks) barely to equal the amount of urine that was formed She received no salt Initially she had a rather high undetermined acid in the serum, presumably mostly ketones, while S was only moderately reduced With this form of therapy the body fluids evidently became somewhat more contracted, as the total base and chloride rose slightly in the first five hours The ketones rapidly diminished, but S also decreased Therefore, although its excretion was not measured, it can be inferred that base was excreted CO_2 rises because of loss of fluid and also because of the base liberated from ketones and not excreted During the next five hours the patient received slightly more fluid, but still an insufficient amount S is maintained, the undetermined acid fraction is still further reduced and CO_2 augmented by an equal amount In brief, dehydration

is not overcome and the increase of CO_2 that is observed is due to replacement of the ketones that have been burned and to fluid depletion   The excretion of chloride is very small, 3 m Eq   Although base excretion was not quantitatively determined, the ketones in the urine, evidenced by a heavy nitroprusside reaction, probably carried base with them   Depletion of electrolytes must have continued throughout the period of study in this individual, but its effects on the serum were masked by the simultaneous loss of fluid   The increase of bicarbonate consequently gives a speciously favorable impression of the efficacy of a therapeutic course which was obviously unsatisfactory if its aim was the restoration of a normal internal environment

2   Treatment with fluids alone   (Cases 81042, 29176–1)

There are no cases that received fluids entirely without salt   The cases listed in this group received less salt than they excreted   Case 81042 was given 2120 cc of fluid and only 46 m Eq of chloride during a period of eighty-two hours in which she excreted 975 cc of urine containing 86 m Eq of chloride   No figures for the excretion of base are available   The serum proteins drop from 8 09 per cent to 6 92 per cent, evidencing adequate hemodilution   The CO_2 concentration rises because of displacement of ketones   Total base and chloride fall, not only because dilution takes place, but because chloride at least was lost in the urine   Base falls farther than chloride because, since its concentration is higher in the serum, it is more affected by dilution   This phenomenon is discussed in detail elsewhere   This therapy causes dilution of the electrolytes   Any augmentation of CO_2 which occurs is referable only to the base liberated by burning ketones   Again, consideration of bicarbonate alone gives an erroneous impression of the results of therapy, and especially of its effects on base

3   Treatment with varying amounts of salt and fluid   (Cases 90339, 53990, 81832, 23514, 60404, 71954–2)

This has been the accepted mode of treatment and most of the cases reported fall within this group   The diagrams of individuals exhibit responses of great variability because of differences in initial electrolyte and fluid depletion, the proportions and amounts of salt and fluid given, the amount of vomiting and nausea and the quantities of fluid and salt excreted

Case 90339 had in the serum an initial chloride concentration of 97 m Eq with a total base of 142 8 m Eq   During the first twenty-two and three-quarter hours she received (4792 cc) more fluid than she excreted   Her salt intake was 548 3 m Eq of sodium, 28 8 m Eq of potassium and 535 2 m Eq of chloride   She excreted 90 7 m Eq of sodium, 51 6 m Eq of potassium and 93 7 m Eq of chloride   With this

therapy the serum chloride concentration rose to 106 m Eq   Serum CO_2 rose because bicarbonate replaced the ketones that were burned and S also increased slightly   This is not easily apparent from the concentrations observed, but becomes so when the extent of original fluid loss, which is represented in the diagram, is considered   During the ensuing twenty four hours she received 1873 cc of fluid more than she excreted, together with 286 m Eq of sodium and 283 m Eq of chloride, of which she excreted 52 and 107 8 m Eq respectively   At the end of this time her chloride had risen to 118 m Eq   notwithstanding further dilution   Base was now normal, 150 2 m Eq CO_2, however, had only risen 3 m Eq and was still very low, 17 8 m Eq   Despite the distorted electrolyte pattern observed at the end of the study, the patient, aside from exhaustion, had felt entirely well for more than twenty four hours

Case 53990 on admission was vomiting profusely and was markedly dehydrated   Vomiting and nausea ceased soon after therapy was instituted   During the first three and one half hours he received 2580 cc of fluid with 258 m Eq of sodium chloride   Although the ketones diminished, CO_2 fell, presumably because of the continued heavy excretion of the former together with a certain amount of base   In the recalculated diagram it is observed that S fell 8 m Eq , whereas total base fell 4 8 m Eq and chloride rose 3 4 m Eq   The amount of sodium chloride added to the serum was apparently insufficient to replace the base and ketones lost   During the next nine hours very little sodium chloride was given, 34 m Eq   Despite slight retention of fluid, chloride and total base rose rapidly in a parallel fashion CO_2 was increased by further elimination of ketones   Because of the low intake during this period these changes can be interpreted only as the result of absorption of fluid from the gastro intestinal tract

Case 81832 was also given large amounts of salt and fluid   In this case salt depletion was not extreme   The effect of the treatment was to cause dilution of the serum with a marked increase of base and chloride   If the observed changes in the electrolytes in this case are recalculated with consideration of the dilution indicated by the diminishing serum proteins, it is found that equal amounts of sodium and chloride have been retained   The increase in CO_2 has been almost entirely at the expense of ketones   In Case 71954–2 the initial serum chloride is high, but base is very low, as are the undetermined acids   In the course of thirteen and one quarter hours he received 4010 cc of fluid more than he excreted and retained 505 m Eq of chloride   The large amount of fluid given caused extreme dilution of the serum   Base slowly rises   Chloride, after an initial rise, returns almost to its original concentration   Unfortunately, no base excretion figures are available, but, as S slowly increases, chloride must have been excreted in equal or greater amounts than base   Because chloride concentration in serum is greatly less than base an excretion of

equal amounts of each will cause a relative increase in serum base concentration

The administration of both sodium chloride and water permits restoration of base and body fluids   Hyperchloremia often results and bicarbonate may remain low   However, these abnormalities of the electrolyte pattern do not seem to have any deleterious effects as far as one can judge from objective and subjective clinical signs

4   Treatment with fluid, sodium chloride and sodium bicarbonate   (Cases 29176–2, 71954–3, 85138, 29176–3)

Sodium bicarbonate was given to four cases, but no conclusive results were obtained   Case 29176 during the first four hours received 119 m Eq of sodium bicarbonate, 285 m Eq of sodium chloride and 2650 cc of fluid   Serum base rose from 141 to 151 m Eq , chloride from 93 2 to 95 6 m Eq   S rose from 23 8 to 36 3 m Eq , but CO_2 rose only from 6 7 to 12 0 m Eq , indicating a very considerable increase in the undetermined acids   During the next four hours no more bicarbonate was given Further dilution took place   Base concentration fell, as did S, but the undetermined acids were greatly reduced, so that CO_2 rose   The chloride concentration rose to normal, 101 8 m Eq   These changes become more comprehensible in the upper figures, where body fluid changes are taken into consideration   It is then apparent that during the first four hours CO_2 increases because of an accession of total base   During the second four hours CO_2 replaces the ketones and total base and chloride are added in about equal quantities   The reason for the increase in ketone acids after bicarbonate administration is obscure   It is known that bicarbonate will increase the excretion of ketones and in this case qualitative ketones persisted in the urine for a longer period than was expected It is conceivable that the ketones came from the cells in response to the addition of base to the extracellular fluids

In Case 71954–3, 107 m Eq of sodium bicarbonate, 247 m Eq of sodium chloride and 3800 cc of fluid were given in eight hours   He developed a hyperchloremia and the concentration of CO_2 rose only by virtue of the base liberated by oxidation of ketones   This patient was vomiting on admission and nausea continued for more than three hours That he then absorbed from his stomach considerable free hydrochloric acid, part of which had been neutralized by the small amount of bicarbonate given, would provide a plausible explanation of the data   In Case 29176–3 much the same phenomena occur   In the last period of four hours 71 m Eq of sodium bicarbonate without any chloride were given   During this four hours chloride and base rose   The concentration of CO_2 increased from 10 8 to 20 5 m Eq , but the concentration of undetermined acids fell from 20 7 to 6 6 m Eq , so that S actually diminished   The sodium bicarbonate given was more than neutralized

by the contents of the gastro intestinal tract while the individual was nauseated   The vomitus secured for analysis contained 33 6 m Eq of total base and 40 4 m Eq of chloride   Case 85138, in four hours, received 1810 cc of fluid, 155 m Eq of sodium chloride and 95 m Eq of sodium bicarbonate S remained constant   The recalculated diagram shows that equivalent amounts of total base and chloride were added to the serum   Undoubtedly, sodium bicarbonate was not given in sufficiently large amounts to produce a demonstrable specific effect upon serum bicarbonate

DISCUSSION

The cases are complicated by differences in initial salt and water depletion, the occurrence of vomiting or nausea, and variation in renal function, as well as by the type of therapy used   However, certain inferences can be drawn from analysis of the data   Treatment with carbohydrate and insulin alone is unsatisfactory   It does not supply the elements that have been wasted by the body   Dissipation of saltless water by means of insensible perspiration and of salt and water through renal activity and vomiting continue   These processes lead to a concentration of all the solutes in the interstitial fluids   If the initial loss of salt is small, the concentrations of serum electrolytes may appear surprisingly normal unless the degree of body fluid loss is considered   Treatment with fluid without salt is also unsatisfactory   Dilution occurs, making the salt depletion even more apparent   In either instance, changes in the concentration of CO_2 do not indicate any essential improvement in the patient's condition

The CO may rise further when no fluid is given because the body fluids become more concentrated   Nevertheless, administration of fluid at least has the advantage of combating, to some extent, dehydration   In both cases increases of CO_2 are derived only from base liberated by combustion of ketones, while the actual base content of the body must suffer loss through urine and vomitus

Since sodium, the major basic ion of intestinal fluids, cannot enter cells, the rising osmolar concentration of extracellular fluids that occurs when no salt and no fluid is given, must force the cells to give up water   Atchley, Loeb, Richards, Benedict and Driscoll (5) showed that during development of the dehydration of diabetic acidosis intracellular as well as extracellular fluid is lost, so that further concentration is even more undesirable   If fluid without salt be given, the osmotic concentration of the extracellular fluids would diminish, forcing the cells to imbibe water or perhaps to lose base. Carried far enough this would lead to complete disruption of the internal environment

The importance of replacing the salt and fluid lost from the body has already been discussed in a previous article (3) in relation to shock and to the continuance of the stupor and other clinical symptoms   The rapid

recovery of Case 82529, without any change in the concentration of serum bicarbonate, under the influence of parenteral administration of normal sodium chloride solution, was cited

Three cases have been cited in which salt and fluid in varying amounts produced electrolyte patterns that were distorted and yet were more in keeping with a normal internal environment than most of the patterns resulting from either of the other forms of therapy discussed   Hyperchloremia with a continuously low CO_2 concentration is frequently observed   This is shown strikingly in Case 90339   After forty-seven hours the serum of the patient had a chloride concentration of 118 m Eq and a CO_2 concentration of only 17 8 m Eq   The concentration of her serum base was, however, normal, 150 6 m Eq   That this distortion is caused by a dilution of the serum with sodium chloride solution has already been pointed out   In diarrhea of infants, in which base is lost in excess of chloride, Hartmann (6) has claimed that treatment with sodium chloride and water alone is unsatisfactory because it gives rise to hyperchloremia and still further depresses bicarbonate   However, the depression of bicarbonate caused by sodium chloride is not due to displacement of one acid by the other, but to dilution of the bicarbonate by chloride solution   Hoag and Marples (7) have further pointed out that hyperchloremia occurs only when the amounts of chloride solution given are too small to ensure an adequate flow of urine   The effect of renal activity is shown by Case 71954-3   During the first eight hours the serum chloride concentration rose from 98 5 to 106 6 m Eq , although the base concentration remained constant at 140 m Eq   During this period the excretion of total base, 22 2 m Eq , almost equalled the excretion of chloride, 24 9 m Eq   Although his CO_2 concentration at this time was only 14 3 m Eq the patient was entirely asymptomatic   During the next sixteen hours 184 1 m Eq of chloride were excreted with only 31 1 m Eq of base   Unfortunately the electrolyte concentrations were not determined at the end of this time

The presence of an extremely small chloride excretion at the time when hyperchloremia exists in the serum is also illustrated by Case 60404 Serum chloride concentration rose from 95 2 to 105 m Eq in five hours During this period 260 m Eq of chloride were administered but only 8 m Eq were found in 556 cc of urine   Extraordinary serum chloride concentrations are seen after clinical recovery   In Case 23514 the final chloride concentration was 120 7 m Eq   Hartmann and Smyth (8) found that in pyloric obstruction, when chloride became depleted, patients, in spite of the retention of abnormally large amounts of CO_2, excreted acid urine and thus maintained a normal concentration of base in the body fluids   In recovery from diabetic acidosis, under administration of sodium chloride, the reverse obtains   Until a normal base concentration is restored, the excretion of chloride remains low, even when serum chloride has been driven to an exceedingly high level

Despite the low CO_2 concentration and the existence of hyperchloremia, the patients are entirely free of symptoms, provided that fluid and base requirements have been satisfied In no case has the actual minute volume of respiration been determined, but in all cases which have progressed to this point, all visible hyperpnea has disappeared, although the bicarbonate concentration may be still as low as that observed in patients who, during the development of acidosis, suffered from extreme air hunger The maintenance of a low alveolar CO_2 tension with constant CO_2 production must, undoubtedly, necessitate increased breathing However, even a 50 per cent reduction of CO_2 tension, once established, requires only 100 per cent overventilation, a degree of hyperventilation that is hardly noticeable Furthermore, during recovery the CO_2 tension is rising It would seem that the restitution of volume and ionic concentration of the body fluids is of prime importance, whereas, the replacement of individual anions to recreate the normal pattern is of secondary importance This rearrangement may not occur until long after the disappearance of all untoward symptoms and reactions Field (9) and others have asserted that the bicarbonate deficit itself inhibits glucose utilization and have advanced this as a reason for administration of bicarbonate In physiologic or clinical studies no basis for such an assertion is to be found It has been demonstrated that administration of acidifying salts does not impair oxidation of sugar (10) In fact, Haldane (11) and others claim that it has an accelerating action on such oxidations In other diseases with comparable acidosis, for example, nephritis, carbohydrate combustion seems not to be affected Furthermore, it has been observed in the present series that utilization of sugar during recovery from diabetic acidosis may advance to the appearance of hypoglycemia while bicarbonate is still greatly depleted In patient 79156 the blood sugar fell in successive observations from 427 to 277 to 117 mgm per cent with corresponding bicarbonate values of 3 2, 6 4 and 9 7 m Eq During this period of 6 5 hours he received more than 80 grams of sugar There can be no doubt that he was burning carbohydrate rapidly at the time of the second and third blood examinations, although the bicarbonate was still at levels usually associated with coma

Hartmann and Darrow (12) have presented a more logical argument for bicarbonate administration It is evident from the serum electrolyte patterns at the height of acidosis that the concentration of S is, in many cases, diminished The actual quantity of S must, of course, be even more depleted by loss of body fluids That hyperchloremia develops when sodium chloride solution is given in the attempt to replace this deficit has already been demonstrated In order, then, to restore the normal electrolyte pattern as speedily as possible, administration of bicarbonate would seem advisable However, dilution of the extra-

cellular fluids with sodium bicarbonate solution alone would cause greater distortion of the pattern than would dilution with sodium chloride, because of the difference in concentration of chloride and bicarbonate found in normal serum   Hence, a combination of the two salts should be given   It has already been stated that the amounts of sodium bicarbonate that were given to patients in this series were too small to permit any adequate deductions

To be effective, large amounts of this salt would have to be given Administering the salt by mouth is usually impossible because of the frequent occurrence of nausea and vomiting   This necessitates discontinuing all oral feeding and resorting entirely to parenteral administration of fluid, carbohydrate, and salts   The time required to prepare a solution of sodium bicarbonate for intravenous use precludes its use until the patient should be well on the way towards recovery, if large amounts of fluid and sodium chloride are given   Cunningham and Darrow (13) have proposed a solution made by mixing suitable proportions of sodium bicarbonate and hydrochloric acid   This solution, however, contains but 30 m Eq of sodium bicarbonate per liter, an amount too small to evoke an adequate effect   Hartmann and Senn (14) have proposed using normal sodium lactate solution in amounts varied according to the estimated needs of the individual   This solution provides a means of giving an adequate amount of base to combine eventually with CO_2 However, the solution also is not immediately available because it must be given intravenously and, therefore, cannot be made in advance if nonspecific reactions are to be avoided

Although it may be desirable to give sodium bicarbonate or a substitute, this salt will not furnish all the requirements of the depleted body fluids   Chloride has been lost in large amounts   The administration of large amounts of sodium chloride as early in the course of treatment as possible is quite essential in accelerating recovery and should not be delayed until a suitable bicarbonate solution can be prepared   .

SUMMARY

The effect, on the recovery from diabetic acidosis, of administering varying amounts of fluid, sodium chloride, and sodium bicarbonate, has been discussed   Treatment with carbohydrate and insulin alone, or with the addition of water only is unsatisfactory   The giving of sodium chloride leads to rapid recovery, even though hyperchloremia develop The individual appears to retain chloride in high concentration until the base concentration approaches normal   Chloride is then excreted in excess of base   Clinical improvement parallels the restoration of body fluid and total ionic concentrations rather than the replacement of individual ions

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TABLE I

Data on blood

Patient	Day after onset	Plasma									Blood sedimentation time
		Total protein	Albumin	Total globulin	Fibrinogen	Euglobulin	Pseudoglobulin I	Pseudoglobulin II	Albumin/Globulin ratio	Viscosity	
		grams per 100 cc	grams per 100 cc	grams per 100 cc	grams per 100 cc	grams per 100 cc	grams per 100 cc	grams per 100 cc			minutes
Case I P G	2	7 26	3 61	3 65	0 79	0 94	1 17	0 75	0 99		115
	6	6 41	3 49	2 92	1 06	0 20	1 06	0 59	1 19		30
	14	6 04	3 35	2 69	0 79	0 19	0 98	0 73	1 24	1 9	97
	36	6 68	4 13	2 55	0 49	0 47	0 91	0 69	1 61	1 5	180+
Case III A K	5	5 61	2 84	2 77	0 27	0 33	0 99	1 20	1 03	2 1	18
	30	6 76	3 07	3 69	0 53	0 38	1 95	0 84	0 81	1 6	155
Case IV F F	11	5 99	2 18	3 81	0 74	0 66	1 31	1 10	0 57	1 8	7
	64	6 56	3 73	2 83	0 61	0 52	1 13	0 59	1 31	1 6	180+
Case VI J B	7	6 55	3 14	3 41	0 59	0 75	1 30	0 77	0 91	2 0	16
	48	6 35	3 12	3 23	0 31	0 38	1 98	0 57	0 96	1 5	180+
Normal amounts		6 67	4 07	2 59	0 29	0 68	0 94	0 68	1 57	1 7	180+

Case II   M B   Female, aged 37   Type III pneumococcus infection, right lower lobe   Approximately 18 hours after the initial chill the amount of fibrinogen had increased to 0 93 gram, the maximum observed in this patient The minimum sedimentation time, however, was not reached until the sixth day when the viscosity was greatest, the fibrinogen diminished but the total globulin increased slightly   During convalescence the amount of fibrinogen and globulin decreased, the viscosity diminished and the sedimentation time increased until normal values were found on the thirty-ninth day   See Figure 1

Case III   A K   Male, aged 28   Type III pneumococcus infection, left lower lobe   In this patient the fibrinogen and total globulin were practically normal in amount on the fifth day of illness, yet the sedimentation time was short (18 minutes) and the viscosity 2 1   During convalescence, on the thirtieth day, both the amount of fibrinogen and total globulin was increased, the viscosity decreased and the sedimentation time was nearly normal   See Table I   Observations in this case corroborate the findings of others who showed that increase in sedimentation time did not always parallel decrease in globulins

Case IV   F F   Male, aged 37   Pneumococcus group IV infection, right upper and lower lobes   The amount of globulin and of fibrinogen was increased together with an increased viscosity and diminished sedimentation time as shown in Table I   On the sixty-fourth day, the viscosity and sedimentation time were normal but the amount of fibrinogen was still increased to 0 61 gram

Case V   S S   Male, aged 29   Group IV pneumococcus infection, four lobes involved   Although the fibrinogen content increased markedly between